CHEMICAL MODELLING

OF

PLANT NUTRIENT SOLUTIONS

A thesis submitted to the
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in fulfilment of the requirements for the degree of
MASTER OF SCIENCE

by
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To My Parents

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SUMMARY

This thesis describes an investigation by computer simulation of some of the factors affecting micronutrient uptake by plants from hydroponic solutions. A computer based, chemical speciation model of plant nutrient solutions has been developed. This consists of about 270 complexation equilibria and 39 potential solids, as well as various redox and atmospheric equilibria.

Various features were brought into the development of the model leading first to the incorporation of stability constant data for plant exudates with various micronutrient metal ions and secondly to the introduction of adjustments aimed at allowing for various kinetic factors, involved in the dissolution of calcium carbonate. Caffeic acid was chosen to represent the exudates as this is the major component in this category that is released by plants. Owing to the paucity of literature data, an experimental characterization by potentiometry of various caffeic acid-metal ion systems was undertaken. Formation constants with caffeic acid in 0.1 mol dm^{-3} (Na)[Cl] at 25°C were accordingly determined and are reported for the metal ions zinc(II), copper(II) and iron(II). Owing to rapid and virtually complete reduction by caffeic acid, constants could not be determined for the iron(III)-caffeate system by the method of potentiometry. In any event the iron(III) complexes are probably of only a transient nature and are thus not of importance as far as the nutrient solution model is concerned. Constants for the manganese-caffeic acid system were also required but these had to be estimated because there was not sufficient time to determine them experimentally. A literature survey of kinetic factors involved in the dissolution of calcium carbonate was undertaken. In consequence , it was found expedient to adopt an empirical adjustment of the relevant constants for incorporation into the model.

By comparing computed with experimentally determined pH's of a number of nutrient and standard buffer solutions, the model was successfully validated for the purposes of this study. The nutrient solution model was thus used to investigate the role of speciation in relation to the important effect of chelation on micronutrient uptake of copper, zinc and manganese by plants from solution. Further, the model was used to simulate the addition of caffeic acid to the nutrient solutions and inferences were made regarding the bioavailability of these three metals and iron to plants. Particular attention was focussed on iron, and the effects involved in the iron stress response shown by certain plant species. Finally, various anomalies reported in the literature were considered. These are discussed and some ideas are proposed on the mechanisms involved in plant nutrition.

LIST OF SYMBOLS

A : Debye-Hückel constant, equa. 2.6

a : activity, defined by equa. 2.2

$a(i,j)$: stoicheiometric coefficient of component j in complex i, equa. 3.11

$\mathring{a}_i$: ionic size parameter of the ith ion, equa. 3.10

B : Debye-Hückel constant, equa. 3.10

β_{pqr} : overall stoicheiometric formation constant, equa. 2.3

C : empirical constant, equa 3.10

C_i : concentration of the ith complex, equa. 3.10

E_{const} : sum of all constant potentials, equa. 2.11

E_{Cell} : measured EMF of the cell in volt, equa. 2.10

Eh : reversible redox potential of the solution, equa. 3.6

ϵ : convergence criterion, i.e. a small positive user specified number, equa. 3.13

$\Delta G_m^{\ominus}$: standard molar Gibbs free energy of reaction, equa. 3.2

γ_i : activity coefficient of the ith ion, equa. 2.6

H_T : total proton concentration (mol dm^{-3})

ΔH_m^o : standard molar enthalpy of reaction, equa. 3.4

I : ionic strength (mol dm^{-3}), equa. 2.5

K : stepwise stoicheiometric formation constant

K' : conditional formation constant

K_{sp} : solubility product

K_w : dissociation constant of water, equa. 2.7

L_T : total ligand concentration (mol dm^{-3}), equa. 2.9

M_T : total metal concentration (mol dm^{-3})

Q_γ : quotient of activity coefficients, equa.2.4

$\dot{R}$: natural gas constant = 8.314 $JK^{-1}mol^{-1}$, equa. 3.1

R : crystallographic R factor, defined by equa. 2.12

: molarity activity coefficient, equa.2.2

S : Nernstian slope, equa. 2.10

$\Delta S_m^{\ominus}$: standard molar entropy of reaction, equa. 3.4

T : temperature in Kelvin

T_i : total concentration of the ith component, equa. 2.11

$\mu_A^{\ominus}$: standard chemical potential of species A in solution, defined by equa. 3.1

X_j : concentration of the ith component

Y_j : difference function of total component concentrations, equa. 3.12

$\bar{Z}$: complexation formation function defined by equa. 2.8

$\bar{Z}_H$: protonation formation function defined by equa. 2.7

z_i : charge on the ith ion, equa. 2.5

Abbreviations used for the ligands in this study

cit : citrate

DTPA : diethylenetriaminepenta acetic acid

EDDHA : ethylenediaminedi(o-hydroxy phenyl acetic acid)

EDTA : ethylenediaminetetra acetic acid

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Plant Nutrition

Plants are obviously of tremendous importance to life on earth. This is because they can by the unique chemical process of photosynthesis convert water into oxygen using energy in the form of light, and together with carbon dioxide form simple carbohydrate sugars. Both of these products are essential to most forms of life as we know them today. With the increases in the world population a function of exponential growth, and even now thousands of people dying from malnutrition and starvation, agricultural crops are becoming increasingly more important. Much renewed interest has therefore been focussed on the fertilization and, from the scientific point of view, factors affecting nutrition of plants (115, 124, 139).

Plant experiments have been conducted for more than a century using hydroponic solutions (81, 136). This represents the technique of growing plants without soil and provides for a much greater degree of control of the elemental environment surrounding the roots. This has facilitated the determination of the essential micronutrients required for normal plant growth, as defined by Arnon and Stout (136). Furthermore, it has become apparent that adequate growth does not only rely on the total concentration of the essential elements but nearly always on the concentrations of their bioavailable forms as required by the plant. These concentrations are directly dependent on other components, especially ligands, and the conditions of the system such as pH and reduction potentials. In general the concentrations of the various physico-chemical forms are of utmost importance in aqueous systems as the total concentrations of the elements do not provide information about their bioavailability (66).

Unfortunately trends observed in plant experiments are not always easy to discern as they very often differ from one plant to the next. In some instances, this is attributed to genetic variations (137, 143). Furthermore the system of plants growing in soil is immensely complex, with a multitude of different factors having an effect on their growth (81). Thus observed trends caused by changing factors are often difficult to interpret. This is another reason for studying plants grown in soilless cultures as better control of changing experimental factors can be maintained. The nutrient solution can thus act as a model of the soil solution normally bathing the roots of plants growing in their natural environment. Therefore it is proposed that the study of plants grown in nutrient solutions can lead to a better understanding of the processes involved in the uptake of nutrients. It must be borne in mind, however, that not all of the trends observed with nutrient solutions apply to plants grown in soil. An example of this is observed in the nutrition of zinc(II) (120). It has been shown that zinc deficiency can result from the use of DTPA (5, 121). On the other hand it has been observed that chelating agents applied to soil can increase zinc uptake (141, 116). Similar observations have been made in the iron nutrition of plants (124).

Various trace metals are of great importance to life in general and to plant growth in particular (66), even though only minute quantities of these metals are required in some cases. For instance, iron deficiency can lead to severely chlorotic plants with greatly reduced yields, as this element is involved in various biochemical redox processes acting as an electron carrier (134). Furthermore it has been estimated that 10-15% of the world's population suffers from iron deficiency and is anaemic. As iron is involved in the oxygen transport mechanism of many living beings, this thus constitutes a severe health hazard in some parts of the world (138). The iron nutrition of plants has thus been the subject of a number of investigations over the past few years. Furthermore, iron deficiency is a very complicated disorder (135), affecting plants through a number of factors which include amongst others, the presence of calcium carbonate, high levels of soil phosphate and other heavy metals, genetic variations and added organic matter.

Similarly, adequate zinc nutrition is of importance to plant growth as it has been found that this element is closely involved in many enzymes of importance to biochemical reactions as well as being involved in the synthesis of RNA and DNA (134). As has already been pointed out earlier, its bioavailability to plants is critically dependent on the particular chemical form of the zinc in solution. So it has been postulated in human nutrition that skin diseases observed in vegetarians are not necessarily due to a deficiency of total zinc, but due to a deficiency of the zinc-methionine complex found mainly in meat (134). Similarly, manganese and copper are present in all animal and plant tissues, where they are essential nutritional elements also involved in various enzyme reactions (134). Copper for instance is of importance for the alleviation of arthritic inflamation which is further discussed in section 1.2. So far the importance of trace elements as nutrients has been stressed. It must be pointed out however, that these essential trace metals can by relatively small increases in concentration become toxic to animals and plants (120). Furthermore, the heavier non-essential trace metals such as lead, mercury and cadmium can cause severe poisoning. As metal ions, opposed to other pollutants are not biodegradable, their potential toxicity in a food chain is largely governed by their physico-chemical forms as affected by the presence of inorganic and organic ligands. The speciation in aqueous systems has thus been the focus of a number of computer aided modelling studies (6, 66, 134).

This has therefore led to the interest in the chemical speciation of nutrient solutions with particular reference to the effect of factors such as added chelating agents. These can be seen as strongly binding ligands and thus are expected to significantly affect the physico-chemical forms of the essential metals and thereby, also, their bioavailability to plants (3). Furthermore, certain compounds which are exuded by plant roots are suspected to also have a marked effect on the mironutrient uptake by plants, especially of the important nutrient iron (15). This is an area in the nutrition of plants that is at the present time not particularly well understood.

Three general areas of investigation in plant nutrition can be distinguished, in which factors affecting uptake are being researched. The first of these is involved with the actual mechanism of transport of ions across the limiting membranes of plant roots. Amongst others the following three theoretical models have been proposed to describe the uptake. These are uptake by first electrochemical potentials, secondly Donnan-type equilibria, and thirdly the carrier theory (1). The second area of study is that of ion mobility in solution, i.e. transportation by convection and diffusion of the nutrients to the root wall and interception by root growth through the soil. Reference (2) gives theoretical treatments of the aspects involved herein. Lastly, research can be directed towards investigation of the chemical speciation of the nutrients in soil solutions or in particular of that in hydroponic solutions. This is undoubtedly an area of prime importance in the study of plant nutrition, for the reasons given above, as this is an investigation of the bioavailable forms of the various nutrients required by plants. Speciation is also of importance to the mobility of ions as it has been suggested that chelation aids transport of the nutrient to the root (3). Thus it was decided to investigate in the present study, correlations between computed speciation in nutrient solutions and published experimentally determined factors affecting uptake of nutrients by plants.

A nutrient solution is a multicomponent system which can result in a large number of possible species and potential solids. Their interactions are extremely complex and therefore a computer aided modelling approach is required in this study. Modelling of such multicomponent systems as proposed here requires the use of large high speed computers and appropriate computer programs and has thus only in the last two decades become a viable proposition, the pioneering work in this area having been carried out by authors such as Sillén and Perrin and their coworkers (4, 71). This allows predictions to be made of the particular physico-chemical forms in which nutrients are present in solution and the seeking of bioavailable species amongst them.

1.2 Modelling

We would like to begin this section by quoting Jenne (6). " The ultimate goal of all research in general and chemical modeling in particular is additional understanding of processes and events which can under the proper conditions facilitate the improvement of human life physically, emotionally and aesthetically. ...appropriate chemical and biological modeling studies of soil solutions and plant nutrient uptake can assist in increasing markedly both the quantity and quality of terrestrial food production. "

While it is realised that this study by itself can come nowhere near this ultimate goal, it is good to bear this ultimate aim in mind. Thus the main objective of this study is to investigate only some of the important factors affecting nutrient uptake by plants. In particular, attention is to be focused on the micronutrient uptake, to obtain a better understanding of the processes involved in this important area of plant nutrition.

Only with the advent of high speed computers in the last few years has the technique of modelling become an integral part of the scientific method. Compared with the experimental investigations carried out so far in plant studies this is therefore a relatively new area of research. Often models are used to make predictions, on the basis of hypotheses and the manipulation of earlier data, subjected to new sets of circumstances. The predictions are usually not obvious due to the many variables in such a system. If they were obvious a computer based model would not be required (140).

A successful modelling study of nutrient solutions which helped to explain some of the observations in plant experiments has been carried out by Halvorson (5). However this was only for a relatively small system of components with thus a limited scope for changing input parameters to the model. Various other modelling studies, in some cases of considerably large and complex systems have been carried out, which have resulted in many interesting findings. For example Jenne (6) states that the modelling of inorganic speciation of dissolved silver in San Francisco Bay led to the suggestion of further important research of elemental analyses of in particular dissolved sulphide. This was directly due to findings of the modelling study that most of the silver would be associated with sulphide and not with chloride as originally thought. Modelling of sea water by Sillén an co-workers (65) suggested that pH buffering of sea water was not governed by the carbonate equixlibria as originally thought but is primarily maintained by the aluminosilicate clays and micas. Modelling studies of the speciation in biofluids (72, 132, 133) led to suggestions for the application of various drug requirements in control of rheumatoid arthritis and excretion of various elements in cases of trace metal poisoning. In these instances, speciation modelling can be extremely useful as a preliminary study before biological screening takes place. Jackson et al (132) in their successful modelling studies of low molecular weight complexes in biofluids, correlated the biological response of extensive screening data by Sorenson with copper speciation, of neutral and charged copper complexes and free copper aquo ion concentrations. As they could establish the exact form of the copper species they proposed requirements for the future design of copper complexing therapeuticals (133). Secondly the model was used in the prescreening of proposed rheumatoid arthritic drugs. A modelling study has been successfully applied to the chemical speciation of urine (74). This led to an improved understanding of the processes involved in the formation of urinary stones. Furthermore, possible reasons were proposed, from the results of this study, for the effects observed on stone formation in a group of welders who used brazing rods that contained cadmium.

These successful investigations thus lent impetus to the proposed study of nutrient uptake by plants, by a computer aided investigation of the chemical speciation in nutrient solutions. It is furthermore proposed that analagous correlations to those found in the above studies, may be found between experimental observations reported in the literature and the chemical speciation of plant nutrients. This could lead to an improved understanding of the nutrition of plants.

To undertake this investigation, the construction of a chemical model is initially required based on the components making up the nutrient solutions. Stability constants for all possible equilibria and potential solids arising from these components need to be acquired. The approach of modelling the chemical speciation in solutions successfully, relies thus on sufficient data of a high degree of accuracy to be present in the literature. Where a paucity of data is found, it is proposed to investigate the particular system in question by some applicable experimental technique. This is envisaged to be the case in the proposed study of plant exudates.

Thus with the hopefully increased understanding of plant nutrition, gained from this study, some experimental observations can possibly be explained, and ultimately fertilisation of crops can be carried out on a more scientific basis.

To the modelling critics it should be pointed out that a model is not necessarily a multicomponent computer implemented construction. For instance the seemingly very simple Debye-Hückel limiting law can be regarded as a model based on assumptions and giving approximate estimates of some 'real' value of the activity coefficients of species in solution.

1.3 Objectives of the research

Based on the needs for the research into plant nutrition outlined in sections 1.1 and 1.2, the objectives of this study are thus the following:

1. A literature survey is to be undertaken of plant experiments conducted in nutrient solutions. This is to be carried out with the view to ascertaining knowledge of the specific factors related to plant uptake of in particular iron, manganese, zinc and copper, with special reference to the effects of added chelating agents and compounds exuded by plant roots.

2. A computer based chemical speciation model of synthetic nutrient solutions is then to be constructed. This will require selection of the components which should include chelating agents and plant exudates as indicated by the literature search. Based on these a data base will then be assembled of all possible protonation and complexation constants and solubility products.

3. Where vital constants are absent from the literature it is proposed to measure these by a potentiometric investigation using glass electrodes.

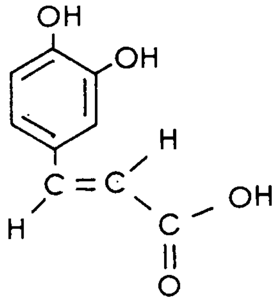
4. The chemical model thus constructed together with a suitable computer program is then to be used to compute the speciation of various specific synthetic nutrient solutions. In particular the simulated addition of chelating agents and plant exudates is to be studied. It is thus proposed to try to correlate the effects of these two factors found on the speciation in solution, with the observations reported in the literature. This could result in explanations pertinent to nutrient uptake by plants in terms of the speciation in solution.

CHAPTER TWO

POTENTIOMETRY

2.1 Introduction

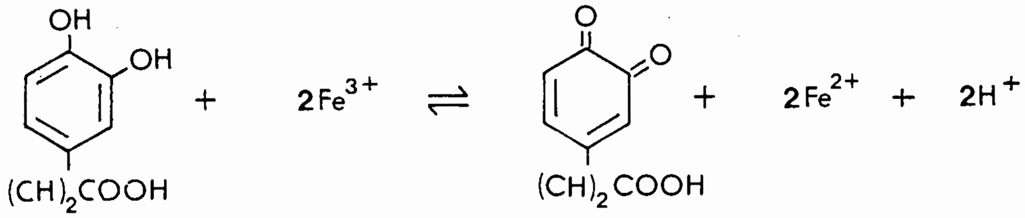
It has been reported that various compounds are exuded by some plants, especially by those species of the 'iron-efficient' variety (7, 8, 9, 10, 13). Under conditions of iron stress, this together with considerable lowering of the pH of the rhizosphere is a manifestation of the so called iron stress response mechanism, which is reviewed in an article by J.C.Brown (15). Results reported by Olsen et al (11) indicate that the major component of these exuded compounds is trans 3,4-dihydroxycinnamic acid known also as caffeic acid. The latter, according to Römheld and Marschner (10) constitutes more than 85% of the exudate. Caffeic acid has the following structure (i) in the fully protonated form :



(i.)

Much evidence has been presented for the 'obligatory' reduction of iron(III) to iron(II) (16) as it has been proposed that the iron(II) aquo ion is the bioavailable form required in the nutrition of plants. Lindsay and Schwab showed that iron uptake is linearly related to the free iron(II) aquo ion concetration in solution above $10^{-9.2}$ mol dm^{-3} (14). Futhermore, they proposed a lower limit of iron(II) concentration required for normal plant growth. This brought about the proposal of two conflicting hypotheses. Firstly, Olsen et al (9), proposed that the reduction takes place in the apparent free space or external solution bathing the roots, through the release of reductants during iron stress.

For caffeic acid the reaction they proposed (11) is the following



They found (17, 18) that various ions such as orthophosphate, hydroxy, copper(II), nickel(II) and to some extent manganese(II) and zinc(II) are effective inhibitors of the reduction process, in that increasing the concentration of these species resulted in the lowering of the iron(II) concentration.

The second hypothesis supported by evidence from various workers (10, 12, 16, 19, 20, 21) suggests the reduction to take place at the outer surface of the plasmalemma at specific sites and to be of an enzymatic nature. Römheld and Marschner suggest that phenols are again operative in this respect (22).

Various of this studies (23, 12, 21) conclude by saying that the phenolics might be important as iron chelators thereby keeping iron in solution and making it available to the plants. As exuded compounds are therefore of considerable interest to the study of iron nutrition of plants, it was proposed to include these equilibria in a nutrient solution model. From modelling results an idea of the likelyhood of one or the other of these hypotheses could then be obtained, based however, solely on the speciation in solution.

Very little work on the caffeic acid system with respect to stability constant determination has however been reported. In fact only one paper appears in the literature (24) containing quantitative stability constant data and this is limited to the two protonation constants and one complexation constant, namely with copper(II). Similarly only one paper was found in the literature that refers to the reduction potential of caffeic acid (25). Otherwise only qualitative speculations or semi-quantitative determinations of the reduction capacity and stability constants have been made in various papers by Olsen and co-workers (23).

The aim then, in this study, was to determine by potentiometry, the stability constants of the complexes formed by the ligand caffeic acid with the series of metal ions zinc(II), copper(II), iron(III), iron(II) and manganese(II).

In deciding upon the titration conditions, the ideal ionic strength of 3.0 mol dm^{-3} was considered (26). It was noted however, that the ionic strength of soil solutions generally approximates to about 0.04 mol dm^{-3} (27). Furthermore, most of the nutrient solutions modelled in this study, Chapter 3, have an ionic strength of 0.015 mol dm^{-3}.

However, it is not good practice to carry out potentiometric determinations in such dilute solutions of the supporting electrolyte since the requirement of constant ionic strength cannot be satisfied. Therefore an ionic strength of 0.1 mol dm^{-3} was chosen as representing a reasonable compromise being appropriate for reliable correction of the determined equilibrium constants to the lower ionic strength values required (28).

Finally, for the purposes of comparison with literature stability constants, an experimental temperature of 25°C was chosen, which is at the same time a reasonable value for conditions of plant growth.

2.2 Theory

The general theory of the potentiometric determination of stability constants has been dealt with in the literature quite extensively (26, 29, 30). Hence only a broad outline of the reactions, equations and assumptions on which this is based will be given here.

Complex forming equilibria in solution can be described by the following general reaction involving ligands (L), metal ions (M) and protons/hydroxy ions (H) :

$$pL + qM + rH \rightleftharpoons L_pM_qH_r$$

where p,q,r are the stoicheiometric coefficients, in the order specified, as used in this study. Electrical charges are omitted for simplicity.

The overall or cumulative thermodynamic stability constant may be defined as :

$$\beta^T{}_{pqr} = \{L_pM_qH_r\} / \{L\}^p\{M\}^q\{H\}^r \qquad ...2.1$$

where { } denote activities.
When r = -1 this refers to removal of protons or addition of hydroxy ions.

The general reaction can therefore take into account formation of protonated, hydroxy, polynuclear and oligonuclear complexes. By convention the equilibria are written here as the formation of complexes from their components, as opposed to dissociation. The stability constants are thus termed formation constants. When considering the (simpler) protonation of a ligand, the term " protonation " constants will only be used, the term " complexation " constants being reserved for metal- ligand and metal-ligand-proton interactions.

Furthermore the cationic species involved in the equilibria are in reality the aquated metal ions or protons eg. $Fe(H_2O)_6^{3+}$, H_3O^+ etc. and the complexation should be regarded as the displacement by the ligand of an unknown number of solvent molecules from the metal ion. The activity of water should therefore appear in the expression of the thermodynamic stability constant (26). It is usually assumed that water is at a constant concentration which is at the same time its standard state. In dilute solutions under STP conditions, the activity is negligibly different from unity and remains unchanged on keeping the activity coefficient constant by means of a supporting electrolyte. This then allows the more elegant, simpler representation of the formation equilibria given above.

Due to problems associated with the measuring of single ion activity coefficients, stability constants are generally expressed in terms of concentration rather than activities, although in the potentiometric determination the activity product, $a_{(H^+)}\,\gamma_{(Cl^-)}$ is measured, resulting in an operational pH (33).

Using $$a = c \times \gamma \qquad ...2.2$$

where a is the activity
c is the concentration in mol dm^{-3} and
γ is the molarity activity coefficient

the overall stoicheiometric or concentration constant for the above reaction is thus defined as

$$\beta_{pqr} = [L_pM_qH_r] \ / \ [L]^p[M]^q[H]^r \qquad \ldots 2.3$$

which is related to the thermodynamic constant by the equation

$$\beta_{pqr} = \beta^T{}_{pqr} \times Q\gamma$$

where $Q\gamma$ is the quotient of activity coefficients of the relevant species :

$$Q\gamma = \gamma_p{}^L \ \gamma_q{}^M \ \gamma_r{}^H \ / \ \gamma_{L_pM_qH_r} \qquad \ldots 2.4$$

For a meaningful determination of the formation constant i.e. for which the β_{pqr}'s remain constant throughout the course of the titration, $Q\gamma$ is necessarily required to be kept unchanged. As the concentrations of reactants change on complexation during the course of the titration, so the ionic strength given by

$$I = {}^1/_2 \sum_{1}^{I} c_i z_i{}^2 \qquad \ldots 2.5$$

changes. From, for example, an extended form of the Debye-Hückel limiting law proposed by Güntelberg (99)

$$-\log \ \beta_i = Az_i{}^2 I^{1/2} \ / \ (1 + I^{1/2}) \qquad \ldots 2.6$$

it is seen that the activity coefficients of the various species are affected by ionic strength which in turn causes changes in $Q\gamma$. Therefore to satisfy the requirement of constant $Q\gamma$, a constant ionic strength needs to be maintained. Thus the titrations are performed in the presence of an excess of background electrolyte which masks the effects of the reactant species and ensures 'constant' ionic strength. The stability constants thus determined are applicable only to the ionic strength and the medium in which they are measured.

Another description of equilibria is that of stepwise formation constants represented by the following set of reactions and equations

$$M + L \rightleftharpoons ML \qquad K_{11} = [ML]/[M][L]$$

$$ML + L \rightleftharpoons ML_2 \qquad K_{12} = [ML_2]/[ML][L]$$

These are related to the overall formation constant by the general expression

$$\beta_i = \sum_{i=1}^{n} K_i$$

To sumaries the values of β_{pqr} and the conditions such as temperature and ionic strength of the solution which affect them, together with the total concentrations of the components M, L and H, determine the type and concentration of the complexes formed.

Various graphical and computational methods for the processing of potentiometric data in order to determine formation constants, are in use, depending on the requirements of the system under study (30, 31, 63).

A useful concept for interpretation of experimental data and determination of protonation constants is the function $\overline{Z}_H$ (or in some texts $\overline{j}$), which is defined as the average number of protons associated with a ligand.

$$\overline{Z}_H = \frac{H_T - [H] + K_w[H]^{-1}}{M_T} = \frac{\sum_{i}^{I} \beta_i [H]^i}{\sum_{i}^{I} i\, \beta_i [H]^i} \qquad \ldots 2.7$$

This is dependent on the free hydogen ion concentration only, being independent of H_T and L_T and can therefore be used to solve the protonation constants, β_i. Similarly, assuming monunuclear binary complex formation only the function $\overline{Z}$ (or in some texts $\overline{n}$), which is defined as the average number of ligands bound to the metal ion and given by the following

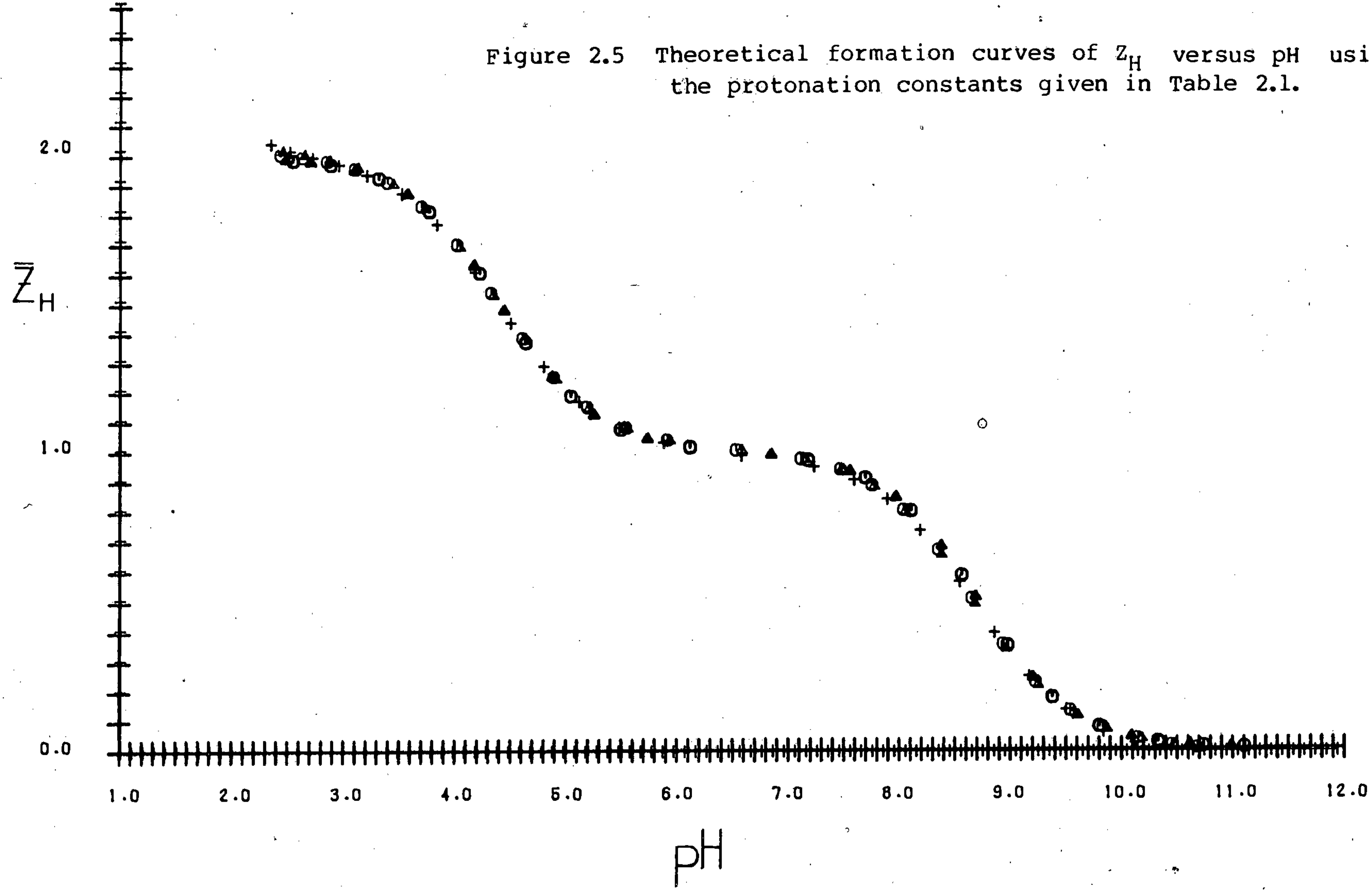
$$\overline{Z} = \frac{L_T - [L]}{M_T} = \frac{\sum_{0}^{I} i\,\beta_i[L]^i}{\sum_{0}^{I} \beta_i[L]^i} \qquad ...2.8$$

This is independent of total metal and ligand concentration and can also, as for $\overline{Z}_H$, be shown to be solely dependent on pH. Therefore a plot of $\overline{Z}$ versus pL (i.e. -log L) known as a Zplot or formation curve is a representation of the equilibria present in solution. Overlapping curves for differrent titration conditions, give an indication of reproducibility and precision. Any deviation for titrations of different metal to ligand ratios from this overlapping pattern is usually an indication of the presence of 'other' complexes, i.e. a fan like appearance is indicative of protonated or hydroxy metal ligand complexes whereas parallel plots suggest polynuclear formation. It is therefore essential that titrations are performed for a range of different metal to ligand ratios (26). If only mononuclear binary complexes are present and overlapping occurs as is usually the case for $\overline{Z}_H$ plots, a rough estimate of the stepwise formation constants can be obtained from the pL and pH values corresponding to the half integral values of $\overline{Z}$ and $\overline{Z}_H$ respectively, using the Bjerrum half Z-bar method (32).

The main computational method used here for the determination of stability constants involves solving the set of mass balance equations at each titration point simultaneously.

Figure 2.4 Experimental formation curves of Z_H versus pH for the protonation of caffeic acid, at 25°C and 0.1 mol dm^{-3} (Na)[Cl].

Figure 2.5 Theoretical formation curves of Z_H versus pH using the protonation constants given in Table 2.1.



The total concentration of each of the components is given by the sum of the concentrations of the individual species and the free component concentration; this can further be expressed in terms of the free concentration of the components and the stability constants. Thus in the case of the ligand the following is obtained :

$$L_T = [L] + [ML] + 2[ML_2] + \ldots + [HL] + [H_2L] + \ldots$$

$$= \sum_{-R}^{R} \sum_{0}^{Q} \sum_{1}^{P} p\, \beta_{pqr} [L]^p [M]^q [H]^r \qquad \ldots 2.9$$

Similarly for M_T and H_T.

The experimental variable determined in the potentiometric investigation using a hydrogen ion ion sensitive glass electrode as in this study is the activity product, $a_{(H^+)}\, a_{(Cl^-)}$ (33). As the chloride ion concentration is known, measured EMF yields a value of $a_{(H^+)} \times \gamma_{(Cl^-)}$. This results in an operational pH, defined by the following equation.

$$E_{cell} = E_{const} + s \log[H] \qquad \ldots 2.10$$

where s is the Nernstian slope $= RTF^{-1} \ln 10$

It should be noted that equation 2.10 is written in terms of the hydrogen ion concentration. This is permissable as the E_{const} term, synonymous with $E_{\ominus}$, incorporates not only all constant potentials but also the hydrogen ion and chloride ion activity coefficients and factors arising from these (34).

As the total analytical concentrations of each titration are known, determination of this one free concentration gives enough information to solve the set of simultaneous equations for the various β's. However this measurement is affected by many diverse factors, such as adsorption of hydrogen ions by the glass membrane, changes in liquid junction potential, sensitivity to metal ions and variation in the standard potential of the glass membrane from day to day due to asymmetry effects, which are difficult to control. Calibration against standard buffers is therefore inadequate. As it is obviously desirable to reduce the influence of the above effects an internal calibration of the electrodes involving refinement of the $E_{\ominus}$ values is therefore carried out in the test solution itself. The protonation data may be processed by the program MAGEC - which can additionally refine any of the other titration variables (34). May et al proposed a procedure for the determination of the protonation constants which was also used in this study and is outlined briefly at the end of section 2.3 in Figure 2.2. Refinement in MAGEC occurs by the minimization of the sum of squared residuals using the simplex method introduced by Nelder and Mead. The residuals represent the differences between measured and calculated EMF's at each titration point.

The computer program MINIQUAD (35, 36) used in this study solves the set of equations by refining both unknown stability constants as well as the the unknown free concentrations at each point of the titrations using the measured free proton concentration. The total number of parameters to be determined is therefore given by

$$(n_{mbe} - 1)n_p + n_b$$

where n_p : the no. of titration points
n_b : the no. of unknown β's to be determined
n_{mbe} : the no. of mass balance equations at each point = the no. of free concentrations

MINIQUAD uses a generalized Gauss-Newton least-squares method for refinement. The sum of squared residuals U, to be minimized is given by the equation

$$U = \sum_{i=1}^{i=m} (T_i^{calc} - T_i^{obs})^2 \qquad \ldots 2.11$$

where $m = n_p \times n_{nme}$ and

T_i is the total concentration of the ith component,

with equal weighting. Linear optimization of shifts guarantees convergence, and refinement is terminated on satisfying two criteria. MINIQUAD also computes a crystallographic R factor, equation given below, used to determine the 'goodness of fit' between experimental and calculated data. This gives an indication of the credibility of the refined 's and the proposed model.

$$R = \left(\frac{\sum_{i=1}^{m} (T_i^{calc} - T_i^{obs})^2}{\sum_{i=1}^{m} (T_i^{obs})^2} \right)^{1/2} \qquad \ldots 2.12$$

Futhermore, the Hamilton test can be used on the obtained R values to decide whether or not a significant difference at the α confidence level exists between various proposed models. If

$$R_i/R_0 < R_{p,n-p,\alpha}$$

where : R_0 and R_i are calculated from equation 2.12 for models H_0 and H_i respectively

p : the no. of unknown parameters and

n-p : the no. of degrees of freedom

α : is the level of confidence

then the alternative hypothesis H_i is not significantly different to hypothesis H_0 (37). In this case the simpler model should then be chosen on the basis of Ockhams razor (38).

Another statistical test computed by MINIQUAD is the value of χ^2 (chi-squared) which gives a measure of how 'normal' or Gaussian the distribution of residuals is. However, not much weight has been given to this test due to the novel way in which it is applied in MINIQUAD. The rather more powerful graphical method of pseudoplotting (39) was used as a final criterion in hypothesis testing. This involves using the refined β's of a proposed model and the relevant experimental conditions to construct theoretical $\overline{Z}$ curves, by using the program PSEUDOPLOT (40). The theoretical curves are then compared with the experimental plots generated by the program ZPLOT (41). For a 'good' model the curves should superimpose well. In this way the possibility of overlooking some complex species is also avoided. Futhermore, the distinction between models with statistically insignificantly different R values is quite often facilitated.

2.3 Experimental

The experimental approach used in this study was similar to that of other workers in the same field and principly involves the potentiometric titration of several solutions containing different metal to ligand ratios, or in the case of the protonation determination different ligand concentrations. The data, obtained in the form of ml of titrant versus mV readings was processed in the manner described in section 2.2.

The caffeic acid used was supplied by Aldrich and was purified by double recrystallization from water. The percentage composition by micro analysis was found to be C, 60.3% ; H 4.5% (and O 35.2%) ; calculations for $C_9H_8O_4$, the non-hydrated form, gave C, 60.0% ; H 4.5% (and O 35.5%). The melting point, with decomposition was at 212-214°C, comparing favourably with a literature value of 217°C (42), but slighty higher than that of 205°C determined by Vorsatz (43).

MAGEC was run on the protonation data and flagged for refinement of the ligand and total hydrogen ion concentration only. This resulted in refined concentrations on average less than 0.2% different to the concentration values input, giving a further indication of the purity of the ligand.

The other chemicals used to make up the solutions were all of at least analytical grade supplied by Merck and BDH. All solutions were made up in glass distilled, deionized water which had been boiled out to remove dissolved carbon dioxide. These were made up to a concentration of 0.10 mol dm^{-3} with respect to chloride ions using sodium chloride (BDH Aristar), for 'constant' ionic strength requirements, see section 2.2, and were prepared by the following methods.

The 0.1 mol dm^{-3} hydrochloric acid was prepared from Merck tritisol ampoules and standardized against borax which had been recrystallized according to the procedure given in Vogel (44). 0.1 mol dm^{-3} sodium hydroxide was prepared under nitrogen from Merck tritisol ampoules and although stored in polythene bottles and protected from carbon dioxide absorption by soda lime selfindicating granules (BDH) was not kept for more than a week. This was standardized with potassium hydrogen phthalate (Merck) and as a further check, also with the standardized hydrochloric acid. Results from the two methods agreed to within 0.1%, a discrepancy which even the most stringent workers (45) regard as satisfactory. The zinc chloride used was prepared as a primary standard from granulated zinc metal dissolved in concentrated hydrochloric acid (Aristar).Due to polarization of the metal surface this was facilitated by a platinum strip thereby setting up an electrochemical cell. The copper chloride solution was prepared using the dihydrated copper(II) chloride salt and standardized by complexiometric titration against EDTA (using murexide as indicator) following the procedure given in Schwarzenbach (46). The EDTA, although made up as a primary standard from its dihydrated disodium salt, was standardized against the zinc(II) chloride solution, results agreed to within 0.2%. The iron(III) chloride solution was made up from a 60% w/v solution (BDH) and standardized against EDTA (46). Testing for iron(II) impurity using the phenanthroline spot-test gave a negative result (47). Various methods were used make up a 'pure' iron(II) chloride solution free from iron(III) contamination; all preparation were carried out similarly under nitrogen to exclude all traces of oxygen and the boiled-out water used was further degassed by bubbling with high purity nitrogen (description of purification follows).

Using freshly obtained tetrahydrated iron(II) chloride salt or iron wire dissolved in concentrated hydrochloric acid (Aristar) resulted in solutions severely contaminated with iron(III), as determined by thiocyanate.

Eventually iron(II) chloride solutions were obtained giving a negative result for the iron(III) spot-test with thiocyanate using firstly refluxed concentrated hydrochloric acid (Aristar) and secondly removing all possible traces of iron(III) oxides by reduction of the iron powder with hydrogen at about 500°C in a quartz apparatus. This solution was stored under slight overpressure of nitrogen and was tested for iron(III) with thiocyanate immediately prior to use in the titrations.

When possible the proton concentration of the metal ion solutions was standardized by titration with sodium hydroxide using the method of Gran (48). End points determined in this way correspond well with those calculated by the titroprocessor. The proposed standardization of the iron(III)chloride solution is somewhat more involved requiring the use of cation exchange resin followed by titration of the eluant with sodium hydroxide. Knowing the iron(III) concentration the proton concentration is determined by difference.

All volumetric flasks and manual piston burettes used were calibrated with deionized water by weighing (allowing, not only for density changes with temperature of the water, but also for the volumetric expansion of the glass with temperature and the archimedes effect on standard weights) (49). Most of the equipment used for the titrations was supplied by Metrohm unless otherwise specified. The titrations were performed in a titration vessel of the mantle type (E8876-20/-50) thermostated to 25°C ± 0.1°C by a HETO thermostat. An automated burette (Dosimat E635), controlled by an automatic titration controller (Titroprocessor E636) was used which also recorded the experimental readings of volume added and EMF measured. Two hydrogen ion sensitive glass electrodes (EA 109) together with a calomel reference electrode (EA 404) in which the saturated potassium chloride solution had been replaced with a saturated sodium chloride solution (Aristar) were used alternately to measure the EMF. The titration assembly together with some of the delivery apparatus is shown in Figure 2.1. All solutions used in the titrations were introduced into the reaction vessel from sealed off resevoirs by manual or automatic piston burettes. These were placed on jacks to avoid disturbing the titration solution by allowing the lowering of the pipes through holes in the cap of the titration vessel to just above the level of the solution. The sodium hydroxide and hydrochloric acid delivery tips were fitted with non return valves as they were actually introduced into the reaction solution. Due to solubility problems experienced with caffeic acid in neutral and acidic solutions, conciderations of electrode immersion and the need for various ligand metal ratios, the use of piston burettes throughout was a tremendous improvement over the usual introduction by pipettes, as there is greater flexibility for designing the titration conditions, as well as allowing for greater accuracy.

Throughout the experiments a carbon dioxide and oxygen free nitrogen atmosphere was maintained in the reaction vessel. The commercial high purity nitrogen was further purified befor being passed over the surface of the titration solution. This was carried out by bubbling the nitrogen through a purification line consisting of 7 wash bottles, which contained the following solutions :

i. 50% KOH solution to remove carbon dioxide,
ii. 30% KOH solution containing 15g pyrogallol per 100ml to remove oxygen,
iii. lead wool in an alkaline KOH (i.e. 200g dm^{-3})

Figure 2.1

Electrode assembly in the thermostated reaction vessel, with the automatic burette and delivery system on the right, and the magnetic stirrer below, as used in this study. On the left is a manual delivery system.

solution containing 1% sodium 1,2-naphthaquinone-4-sulphonate to remove oygen,

iv. excess zinc amalgam in an acidic solution (i.e. 50ml dm^{-3} concentrated H_2SO_4) of chromous/chromic sulphate (300g dm^{-3} $Cr_2(SO_4)_3 \cdot 15H_2O$) to remove oxygen.

The next three bottles were all thermostatted to 25°C.

v. an empty wash bottle,
vi. deionized distilled water,
vii. a 0.1 mol dm^{-3} sodium chloride solution for purposes of vapour saturation.

To prevent back diffusion the nitrogen outlet from the reaction vessel to the atmosphere was passed through a 0.1 mol dm^{-3} sodium chloride solution trap.

A typical sequence for the potentiometric determinations was the following. A protonation titration of a basic solution of the ligand, basic conditions being required for the dissolution of caffeic acid, was carried out with 0.1 mol dm^{-3} hydrochloric acid. This was then followed, on addition of the required volume of metal stock solution, by a complexation determination, titrating with 0.1 mol dm^{-3} sodium hydroxide. In this way the initial data collected during the protonation titration could be used for the electrode calibration in the complexiometric determination which was conducted in virtually the same solution.

The computational procedures employed in the determination of the protonation and complexation constants are represented by the flow diagrams in Figures 2.2 and 2.3, respectively. The diagrams allow only for eventual success, which is obviously not always the case in every study.

Figure 2.2 Flow diagram of the procedure used in the protonation determination.

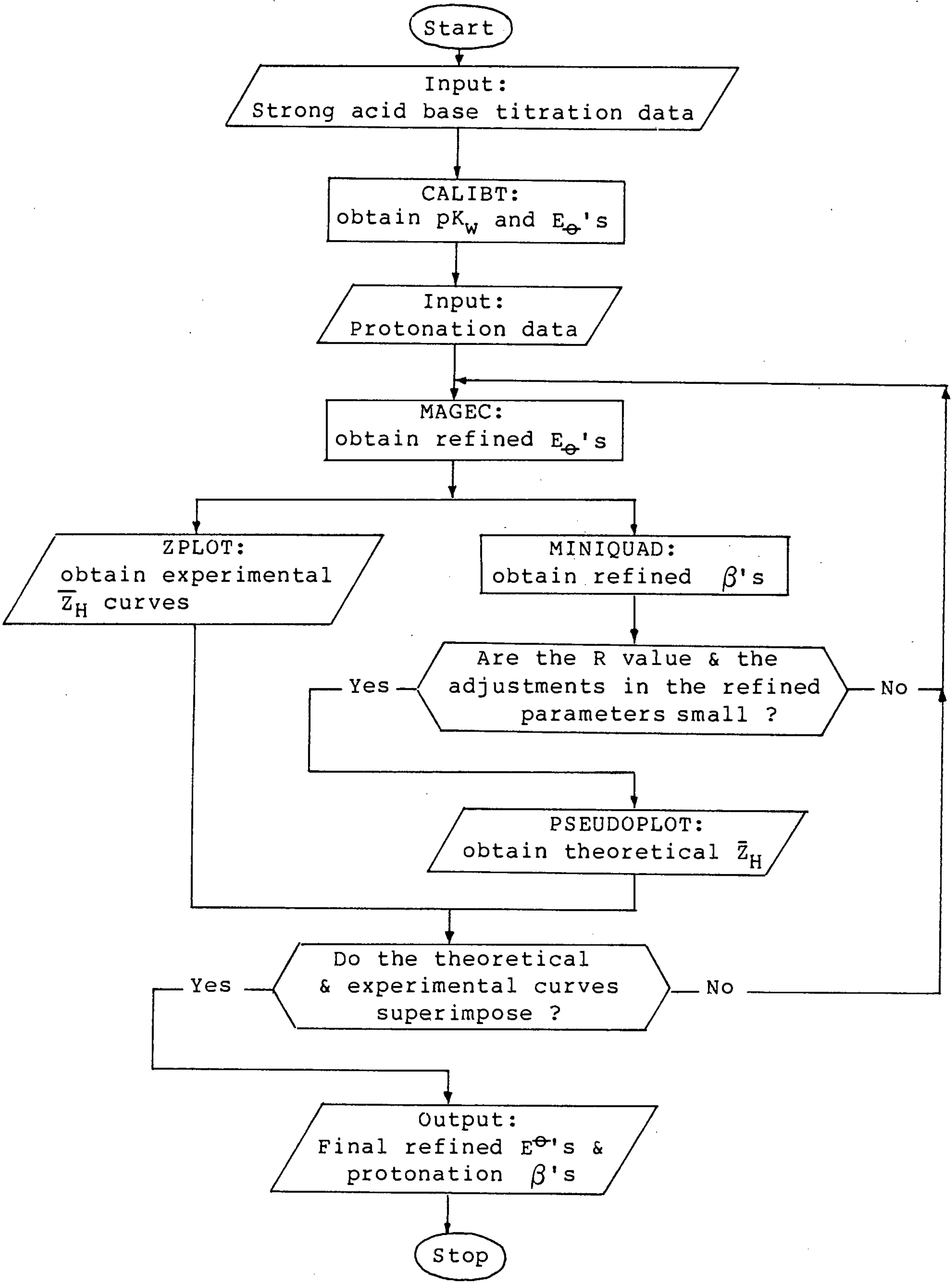
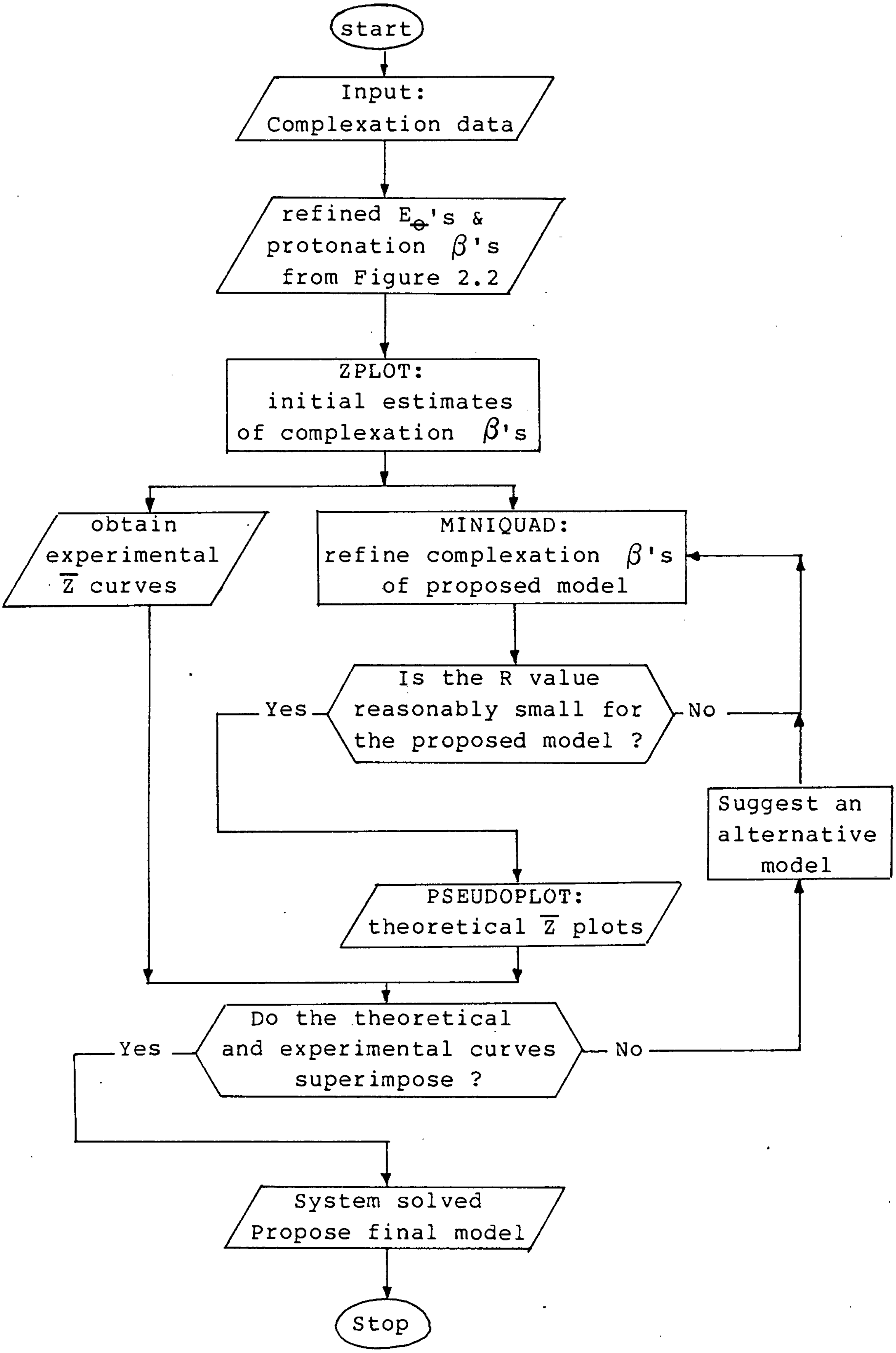


Figure 2.3 Flow diagram of the procedure used in the complexation



2.4 Results and Observations

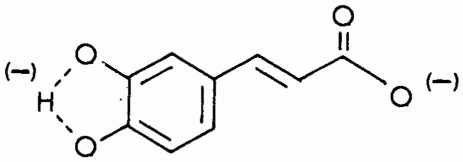
The stabilty constants are reported in the form of overall formation constants β_{pqr}. Whenever it is not absolutely clear what is being meant, or where stepwise constants are used, the relevant equations pertaining to the equilibria will be given.

All titrations were carried out at 25°C ± 0.1°C in a sodium chloride medium of 0.1 mol dm^{-3} ionic strength, and therefore all the determined constants are pertinent to these conditions only. The pH values reported are a rough indication of the regions in which complexation occurred. All concentrations are given as moles per dm^{-3}.

2.4.1 Protonation

No attempt was made to determine the first protonation constant of caffeic acid, (which probably has a pK_1 of about 13 compared with catechol) as the titrations were only run as far as pH 12.

For pH > 12 the ionic strength cannot be maintained reasonably constant at 0.1 mol dm^{-3}. This together with the deviation of the glass electrodes from the Nernstian response, would not allow a meaningful determination. Therefore in this study, although caffeic acid is triprotic, the ligand has been defined as the dianionic species $HCaff^{2-}$, (ii).



(ii)

Table 2.1

Logarithms of the overall protonation constants, β_{10r}, for caffeic acid, determined in this study at 25°C and 0.1 mol dm^{-3} (Na)[Cl],

where $\beta_{10r} = [LH_r] / [L][H]^r$

number of titrations : 7
pH range : 2 to 11.6
ligand conc. range :
0.0038 to 0.0187 mol dm^{-3}
pKw used : 13.76

number of points : 350
χ^2 : 23.9
R : 0.00432
4th cycle of MAGEC,
MINIQUAD

complex 1 0 r	log β_{10r}	SDEV in log β
1 0 1	8.72	0.002
1 0 2	13.13	0.004

Literature Data

complex 1 0 r	log β_{10r}	medium (mol dm^{-3})	ref.
1 0 1	8.76	0.05 KNO_3	24
"	9.07	I → 0	"
1 0 2	13.68	0.05 KNO_3	"
"	13.25	I → 0	"

Incidentally, the very strong basicity of this third proton has been attributed to hydrogen bonding between the two hydroxy groups as indicated in the structure of the ligand (ii) (50). The titrations using two electrodes alternately were replicated several times, titrating in opposite directions with either acid or base thereby indicating the reversibility of the reactions. Different total concentrations of the ligand and different sodium hydroxide solutions were used, which resulted in loss of precision, but hopefully a greater degree of accuracy due to removal of systematic error was thereby obtained. The protonation constants determined in this study are given in Table 2.1, together with the relevant statistics and titration data. For comparison values from the only other formation constants determination of the caffeic acid system reported in the literature (24), are also given in the table. As can be seen from this the constants in this study compare favourably with those determined by Timberlake.

The value for pK_w of 13.76 was obtained from duplicate strong acid, strong base titrations, at 0.1 mol dm^{-3} ionic strength using three glass electrodes and is in good agreement with the values of Dyrssen et al 13.75 at 0.4 mol dm^{-3} (51) and Teder 13.72 (85) at 0.1 mol dm^{-3} ionic strength in the same medium. Refining this β_{00-1} value using MAGEC and MINIQUAD on the protonation data yielded a slightly changed value of 13.78. However in the metal complexation determination, the value of 13.76 was again used as this is in better agreement with the literature. For purposes of comparison and determination of the sites of protonation , the stepwise protonation constants are given in Table 2.2 together with those of some related ligands. Catechol was selected as an obvious model for the catecholic site of caffeic acid, while acetic acid was chosen to represent the carboxylic moeity. Although the constants for 3,4 dihydroxy-hydrocinnamic acid were determined at 30°C they are included as this ligand can be regarded as simply the saturated form of caffeic acid. From this it is seen that K_2 for caffeic acid which is equal to β_{101} by definition of the ligand species, corresponds to protonation of either one of the oxygen atoms making up the catecholic site. K_3 obtained from β_{102}/K_2 represents protonation of the more acidic carboxylic group having a value which compares favourably with log K_1 of acetic acid of 4.56 for similar experimental conditions (87).

Table 2.2

Logarithms of the stepwise stability constants $\log K_r$ for caffeic acid and some related ligands for the purposes of comparison.

Ligand	$\log K_1$	$\log K_2$	$\log K_3$	Medium (mol dm^{-3})	Reference
caffeic acid	not determined	8.72	4.41	0.1 NaCl	This study
	"	8.76	4.49	0.05 KNO_3	24
	"	9.07	4.62	I → 0	"
catechol	13.05	9.229	not applicable	1.0 KNO_3	52
	12.8	9.37	"	0.1 KCl	53
3,4 dihydroxy-hydrocinnamic acid	-11.60	9.36	4.56	[a]0.1 $NaClO_4$	54
acetic acid	4.56	not applicable		0.1 ionic strength	87

---

[a]determined at 30°C.

Comparing the $\log K_2$ values for caffeic acid and 3,4 dihydroxyhydrocinnamic acid with that of catechol, there seems to be the suggestion that the carboxyl group has an electron withdrawing effect on the catecholic site in caffeic acid. This is not apparent for 3,4 dihydroxy-hydrocinnamic acid, which is probably due to the sidechain being fully saturated in this ligand.

The experimental formation curves of $\overline{Z}_H$ versus pH, including for the sake of clarity only every seventh point of four representative titrations with different ligand concentrations are presented in Figure 2.4. These are seen to be very reproducible, which thereby indicates the presence of solely the HL and H_2L complexes. It is seen that the maximum value that $\overline{Z}_H$ attains is equal to 2; this is due to definition in this study of the caffeic acid as a diprotic ligand. Using the set of β's determined together with the conditions of the titrations theoretical $\overline{Z}_H$ versus pH curves were plotted (Figure 2.5) by the program PSEUDOPLOT. These are very superimposable with the experimental plots giving some confidence to the values of the determined β's.

2.4.2 Complexations

By way of introduction to this section we would simply like to quote the following from the paper by D.R. Williams (39) : 'In common with all mathematical treatments of non-simple mononuclear systems we are faced with the qualitative problem of finding the best set of β's and the quantitative task of assigning values to these β's. Qualitatively, the possible β's are limited by the coordination numbers of the metal ions and by the denticity of the ligands.' This indicates clearly the objectives and hints at the possible problems to be encountered in the following investigations.

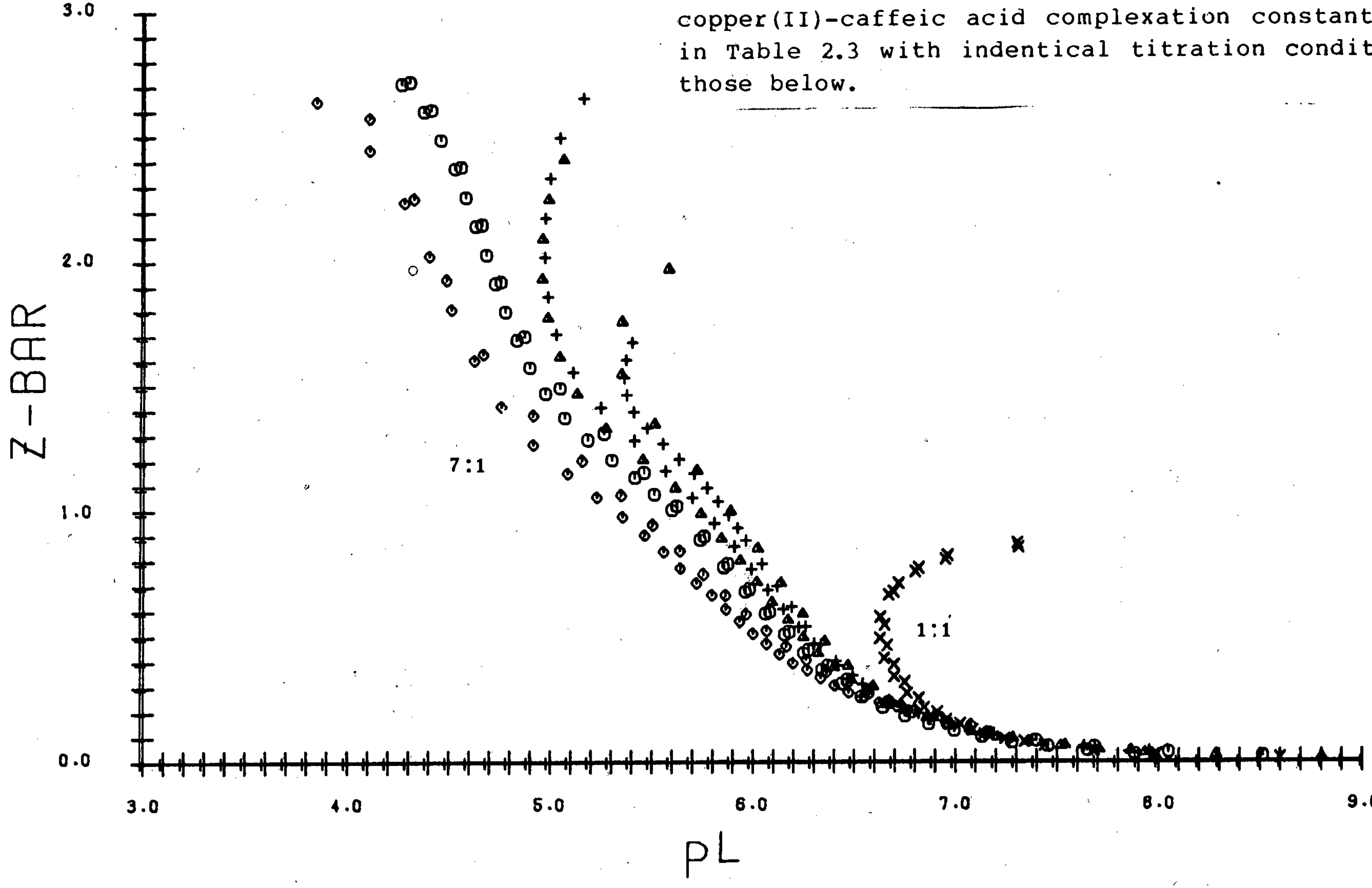
2.4.2.1 The copper caffeic acid system.

A series of titrations of various ligand to copper(II) ratios ranging from about 7:1 down to 1:1 was carried out from about pH 3.3 up to the appearance of a precipitate. These titrations were replicated once or in some cases twice with identical conditions to check on the reproducibility of the system, which was done by plotting the experimental formation curves.

Figure 2.6 shows the experimental $\overline{Z}$ versus pL formation curves obtained from the data of the titrations with ligand to copper(II) ratios of 7:1, 4:1, 3:1, 2:1 and 1:1 only. For clarity these are presented without their duplicates. As can be clearly seen, the plots 'fan out' tremendously, indicating the presence of hydroxy and possibly also polynuclear species. Due to disruption of the hydrogen bonding on metal complexation at the catecholic site, the loss of the otherwise 'undissociable' third proton is facilitated. A distinction cannot be made between the

Figure 2.6 Experimental complex formation curves of Z versus pL for the copper(II)-caffeic acid system, at 25^{o}C in 0.1 mol dm^{-3} (Na)[Cl], for the ligand to metal ratios as indicated.

Figure 2.7 Theoretical formation curves of Z versus pL using the copper(II)-caffeic acid complexation constants given in Table 2.3 with indentical titration conditions to those below.



loss of protons or addition of hydroxy ions. Therefore most of the pronounced 'fanning out' is probably a direct result of the definition of the ligand, i.e. as the dianionic species, $HCaff^{2-}$.

It was rather difficult to determine the exact onset of precipitation. In the titrations of high ligand to metal ratios this was clearly indicated by discontinuities in the formation curves (not shown in Figure 2.6), however this was not shown in the lower ratio titrations. Therefore, for the sake of prudence, only points clearly prior to precipitation were used in the computational determination of the formation constants. This could lead to some complexes being overlooked. Timberlake (24) reports precipitation only at certain concentrations of caffeic acid and copper. However we observed precipitation in all our titrations covering a large range of total concentrations.

The most consistent set of complexes found, which 'best' represented the experimental data, and their respective stability constants as determined in this study are given in Table 2.3, together with literature constants of some related ligands. All the constants were refined over the same titrations using as many experimental data points as possible, being restricted only by the maximum number of input data i.e. 500, as allowed by MINIQUAD. Initially refinements were carried out on the high ligand to metal ratio titrations only, as it was expected that less polynuclear or only mononuclear binary complexation would occur in these. However it was later found that all the proposed complexes were present in sufficient concentrations so that refinement over the whole range of solution ratios could be carried out. The copper(II) hydrolysis reactions, given below, were also incorporated into every trial, with their respective constants at 0.1 mol dm^{-3} ionic strength and 25°C (84, 88), being 'fixed' in the refinements.

$$Cu^{2+} + H_2O \rightleftharpoons Cu(OH)^+ + H^+ \qquad \log K = -7.85$$

$$Cu^{2+} + 2H_2O \rightleftharpoons Cu(OH)_2 + 2H^+ \qquad \log K = -14.52$$

$$2Cu^{2+} + 2H_2O \rightleftharpoons Cu_2(OH)_2 + 2H^+ \qquad \log K = -10.40$$

The 'best' model thus includes three polynuclear complexes, i.e. the pqr complexes 12-1, 32-2 and 23-2, which are of some interest due to their novelty. However, on including these complexes, MINIQUAD would not refine the system for any of the 21r complexes, at least one of which is necessary for the chemical propagation of complex formation. This means that the 32-3 complex does not have any immediate precursors in the system proposed, which can only be justified if the 21r complexes are only present in minute concentrations and therefore insignificant.

The only similar complex of caffeic acid and catechol is the 11-1 complex of which the log β values are in fair agreement.

Table 2.3

Logarithms of the overall stability constants, βpqr, of copper(II) with caffeic acid, determined at 25°C and 0.1 mol dm^{-3} (Na)[CL].

number of titrations : 14
pH range : 3.3 - $\approx$ 7.0
ligand conc. range : 0.0034 - 0.0150 mol dm^{-3}
corresponding $CuCl_2$ conc. range : 0.0038 - 0.0021 mol dm^{-3}
pK_w used : 13.76
number of experimental observations : 473
χ^2 : 73.6
R : 0.002535

complex p q r	log β_{pqr}	SDEV in log β
1 1 1	10.46	0.020
1 1 0	6.02	0.012
1 1-1	0.25	0.008
1 2-1	3.54	0.038
3 2-3	0.97	0.016
2 3-2	7.41	0.024

Literarure Data

Ligand	complex p q r	log β_{pqr}	Medium (mol dm^{-3})	ref.
Caffeic acid	*2 1-2	-11.0	0.05 KNO_3	24
#catechol	1 1-1	0.55	1.0 KNO_3	52
	1 1-1	0.96		53
	1 1-1	0.936	0.15 (Na)ClO_4	56
	2 1-2	-0.944	"	"
	2 1-2	-1.17	1.0 KNO_3	52

*This value is actually for log K_2 described by the reaction

$$CuA^{-}+H_2A^{-} \rightleftharpoons CuA^{4-} + 2H^{+}$$

where A^{-3} is the alternately defined ligand species $Caff^{-3}$

Ligand defined similarly to caffeic acid , i.e. as the monoanionic species $HCat^{-}$

An enormous amount of computing, which is not immediately evident and 'pseudoplotting' was carried out before a decision could be taken in favour of the 'best' model consisting of these six particular complexes. What complicated the decision was the fact that about eight alternative relatively good five to eight membered hypotheses could be found. These all had R values which did not differ significantly on the basis of the Hamilton test (37). Therefore none could be eliminated purely on statistical considerations. On the basis of Ockhams razor (38) however, which states that if there are two hypotheses of equally good fit the simpler should be chosen, all the models with six or more complexes should have been eliminated in favour of the simpler five complex hypothesis. However, using the respective formation constants determined, the theoretical formation curves obtained from PSEUDOPLOT for the five membered hypotheses were somewhat different from the experimental curves and did therefore not represent the experimental data sufficiently well.

Several of the remaining hypotheses were plausible in view of the propagation of formation of complexes. Some required 'fixing' of the constants for one or two complexes during the refinement process. This is not really a very satisfactory approach. On this basis and by Ockhams razor these were therefore eliminated in favour of two six complex hypotheses H_0 and a plausible model H_i. Hypothesis H_0 is reported in Table 2.3 together with literature data of some similar ligands.

The following null hypothesis was therefore set up:

" Model H_0 does not describe the experimental data better than H_i " ...2.1

and together with the following criteria was used to decide between the two possibilities. The R_i value calculated by

MINIQUAD for hypothesis H_i was 0.00394 compared with an R_0 of 0.002536 for model H_0. These give a value for the R ratio ,R_i/R_0, of about 1.55, which is less than the value of 1.89 for the Hamilton R factor, $R_{1000,500,0.05}$, pertaining roughly to these conditions (57). Based on this criterion alone the null hypothesis 2.1 could not be rejected. The χ^2 for H_i is about 170 compared with 70 for model H_0, which at the 5% confidence level with 6 degrees of freedom should however be less than 12.6 (35). Futhermore theoretical formation curves calculated from the refined β's of model H_i, not represented here, showed a less superimposable set of plots compared to the experimental curves, Figure 2.6 , than those of H_0, Figure 2.7. Therefore, mainly on the basis of better superimposability as well as lower χ^2 and R values the null hypothesis 2.1 was qualitatively rejected and model $H_\emptyset$ chosen as the 'best' representation of the data, reported in Table 2.3.

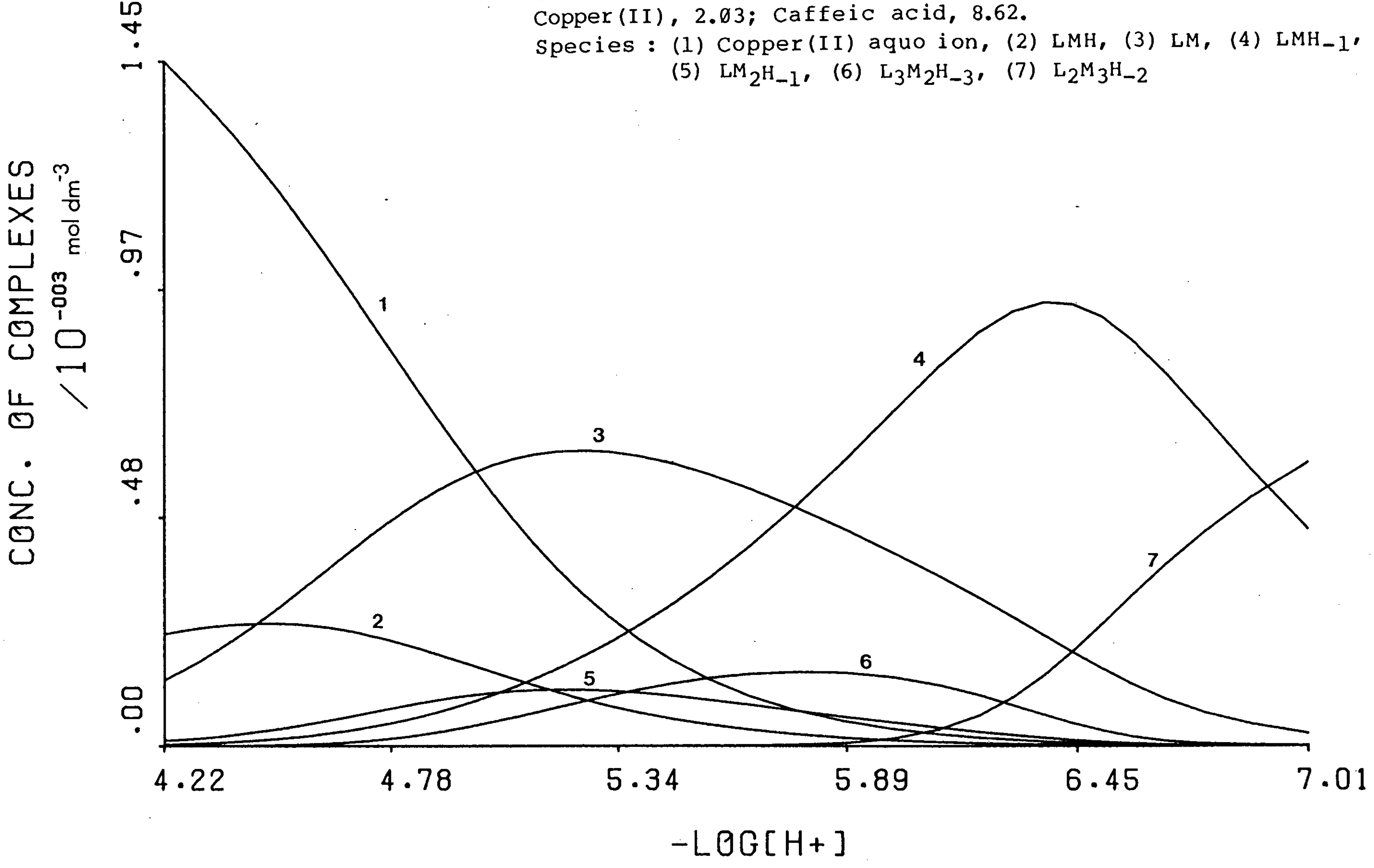
Timberlake reports a stepwise stability constant for the reaction

$$CuA^- + H_2A^- \rightleftharpoons CuA_2{}^{4-} + 2H^+$$

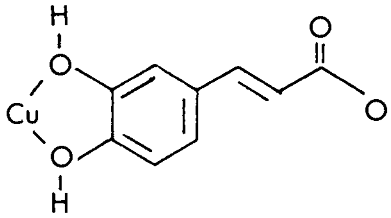
with a value for logK of -11.0, where A^{-3} defines the ligand species caffeate^{-3} . The species $CuA_2{}^{4-}$ corresponds to a 21-2 complex, which may be regarded as an immediate precursor of the 32-3 complex. The 21-2 complex has been included in some of the models tried yielding a log β_{21-2} of -3.76. This gave a logK for the above reaction of -12.7. A distribution diagram for this model showed the 21-2 complex to be present in only insignificant concentration however.

Having decided on the stoicheiometry of the complexes present, what remains to be discussed are the sites at which complexation and protonation can occur, as there are several

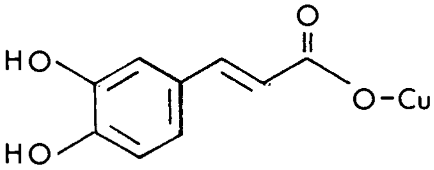
Figure 2.8 Distribution of species in the copper(II)-caffeic acid system.
Total concentration (10^{-3} mol dm^{-3}):
Copper(II), 2.03; Caffeic acid, 8.62.
Species : (1) Copper(II) aquo ion, (2) LMH, (3) LM, (4) LMH_{-1}, (5) LM_2H_{-1}, (6) $L_3M_2H_{-3}$, (7) $L_2M_3H_{-2}$



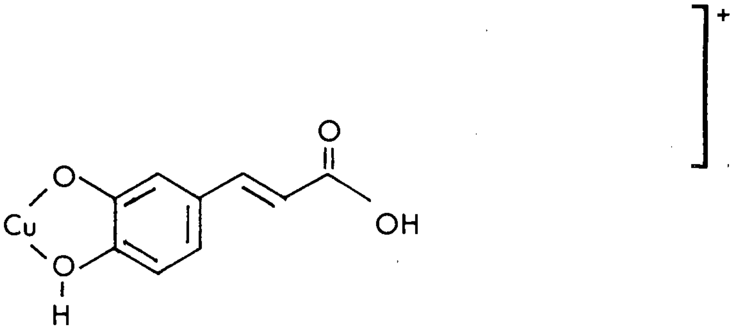
alternative structures for some of the complexes. For instance the 111 complexes i.e. LMH^+ could have one of the following three structures



(a)



(b)



(c)

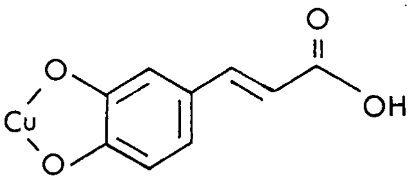
Structure (a) can be ruled out because it corresponds to a 111 complex in the catechol-copper(II) system and this could not be found by Fazakerley and co-workers (56). Calculating the stepwise formation constant from β_{111} and β_{101} for the reaction

$$M + HL \rightleftharpoons LMH$$

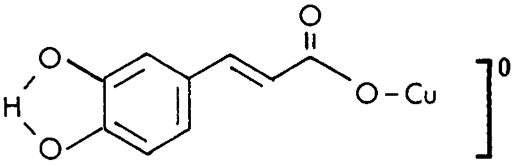
a value for logK of 1.75 was obtained, which is identical to the log of the formation constant of copper with acetic acid (58). This agreement lends support to structure (b).

The correspondence could of course have been fortuitous and possibly (c) is the correct structure. The plausibility of the latter is upheld on considering the pH range over which the 111 complex is significant, as shown in the distribution diagram, Figure 2.8.

Three similar structures to those above could be proposed for the 110 complex. As the corresponding complex was not found in any study of the copper catechol system, this again presents evidence against a structure similar to that of (a). The remaining two possible structures are (d) and (e) given below



(d)



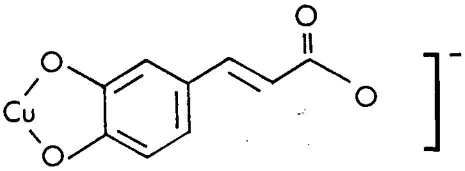
(e)

Complex (d) would be expected to exist in the pH range of 4.41 ± about 2 i.e. for roughly pH 2.4 to 6.4, as is observed in the distribution diagram. On the other hand the log β_{110} value which is equal to logK_1 for

$$M + L \rightleftharpoons ML$$

is 6.02 and is very much greater than about 1.7 as would be expected for complexation at the carboxylic end, see previous discussion of the 111 complex. Futhermore, structure (e) with the deprotonated catecholic site would only be expected to form in the pH region greater than about 6.7. Therefore (d) is probably the most likely structure. Possibly, structure (e) contributes to the total concentration of the 110 complex in the higher pH region.

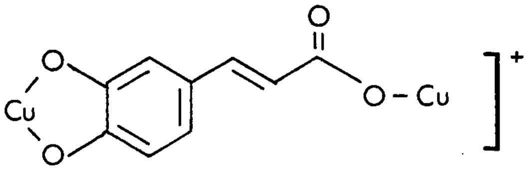
The only really reasonable structure for the 11-1 complex is given by (f) below



(f)

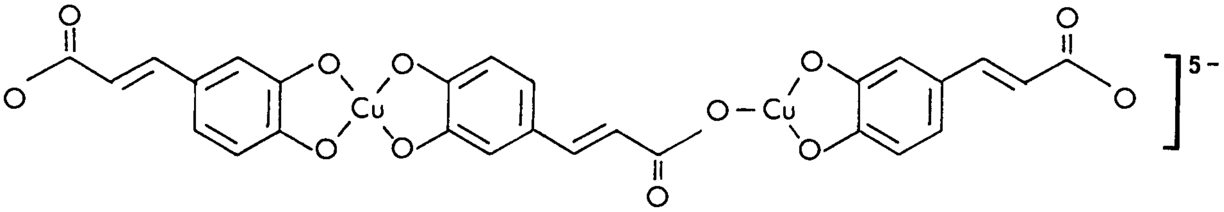
as complexation at the carboxylic end with a completely unprotonated catecholic site is not expected in the pH range in which the complex is observed to be present. Furthermore the value of log β_{11-1} of 0.25 compares quite favourably with literature values of the 11-1 catechol complex, Table 2.3. Thus as catechol has only the one complexation site, structure (f) is probably ths most correct.

The 12-1 complex can only have the one structure (g) due to spatial considerations and is not further discussed



(g)

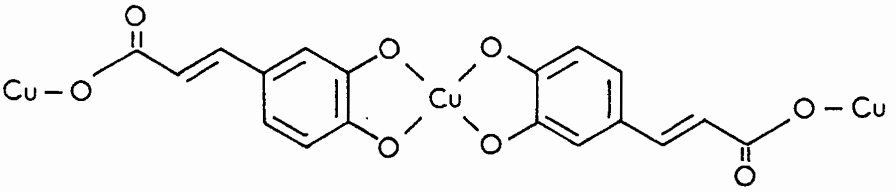
Several structures are possible for the highly charged 32-3 complexes. However,in view of the range of existence of this complex beginning at about pH 5.8, Figure 2.5, the only really likely form is one in which all three catecholic sites are complexed with copper, resulting in structure (h).



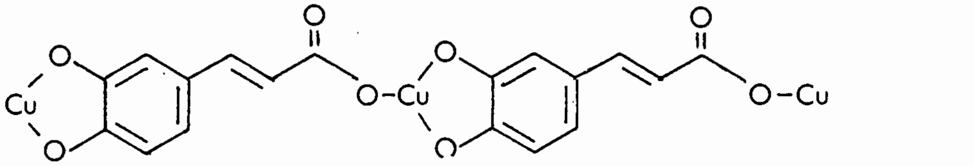
(h)

This could be formed from the 21-2 and 11-1 complexes in a one step reaction, however as mentioned above the 21-2 species was not found to be present in this system as determined here. It could equally be formed from 21-1 and 110, however the precursor, in this case 21-1 is again not present.

The uncharged 23-2 complex has all its sites complexed by copper and could have either one of the two structures (i) or (j).



(i)



(j)

It is difficult to choose between these structures. On the one hand, (i) with the tetragonally distorted square planar arrangement seems more likely at first glance. This could be formed from 12-1 and 110 (e) with the loss of a proton. However structure (e) does not seem all that likely, see above, and therefore the more regular structure (j) is preferred. This could form in two ways, either from 12-1 and 110 (d) with the loss of a proton, or simply from the direct combination of 12-1 and 11-1.

To decide more conclusively on the actual structures of the various complexes in solution, a second method of investigation is required such as NMR. Potentiometry can only be used to deduce the stoicheiometry of a system; proposals for likely structures are largely speculative. What obviously complicates these decisions is the fact that caffeic acid has firstly two sites of complexation and because of this can secondly act as a bridging ligand, at least with copper. This was observed in the similar study of the copper(II) methyldopa system as well (56).

2.4.2.2 The zinc caffeic acid system.

The investigation of complex formation between zinc and caffeic acid was carried out in a similar way to that with copper. The complexation was found to occur in a slightly higher pH region than was found for the previous case i.e. from about 5 to 8. This allowed higher ligand to metal ratios than could be achieved in the determination with copper and therefore a series of titrations with ratios from 10:1 to 1:1 was carried out, again at 25°C with 0.1mol dm^{-3} ionic strength in a sodium chloride medium.

Once again each ratio was replicated with an identical titration and on the basis of the formation curves very good reproducibility was achieved. No precipitation occurred, except early on in the titrations with a 1:1 ligand to metal ratio and these were therefore disregarded. The white precipitate that formed, may have been zinc oxide as seen in the standardization titrations of the hydrogen ion concentration of the stock solution.

The formation curves of the caffeic acid zinc titrations with ligand to metal ratios of 10:1, 5:1, 4:1, 3:1 and 2:1 are given in Figure 2.9 and again show a large degree of 'fanning'. Refinement was again carried out over all the titrations. Included in every trial were the zinc hydroxide equilibria given below, together with the logarithms of their stability constants at 0.1 mol dm^{-3} ionic strength and 25°C (84, 88).

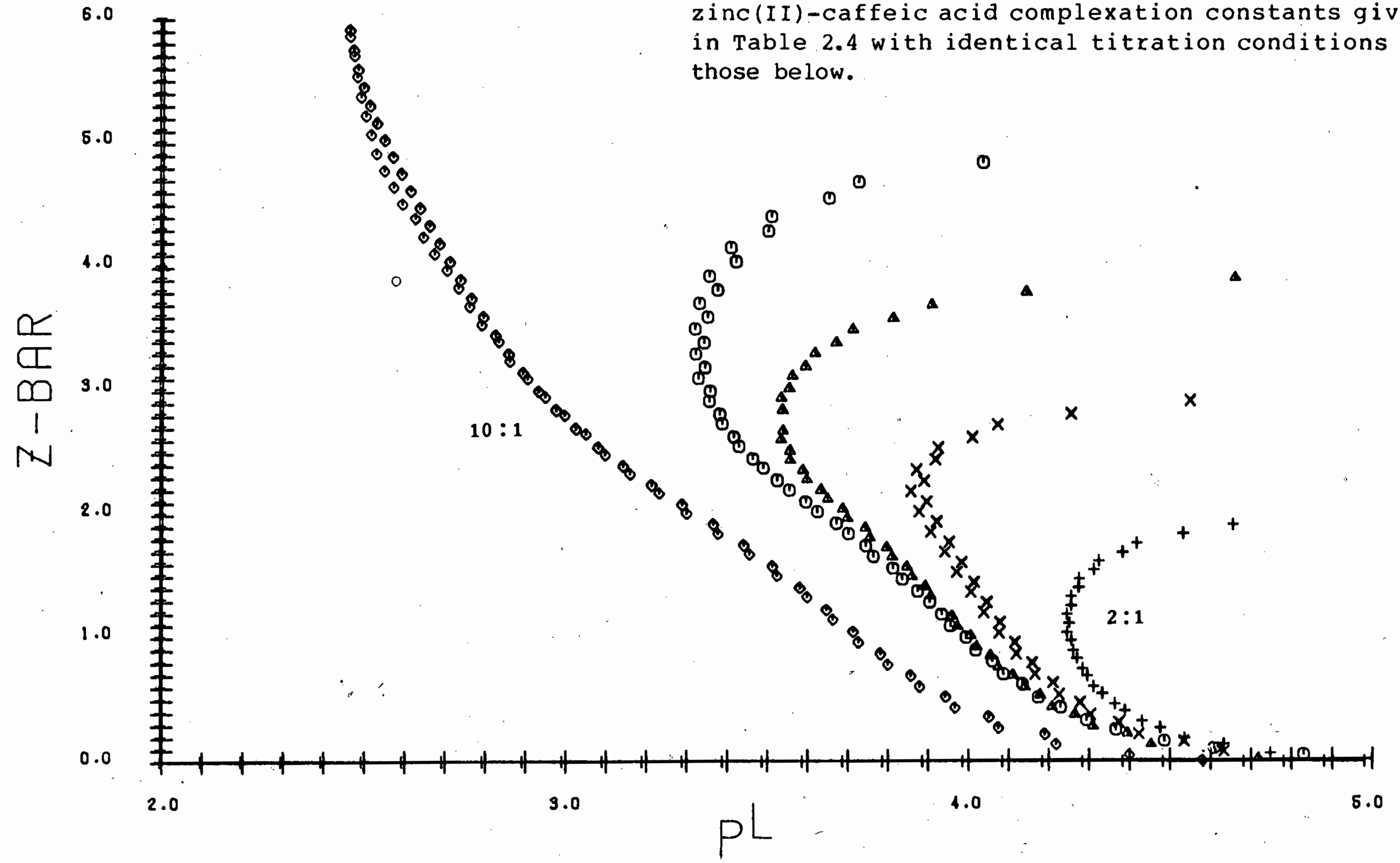
$$Zn^{2+} + H_2O \rightleftharpoons Zn(OH)^{+} + H^{+} \qquad \log K = -9.15$$

$$Zn^{2+} + 2H_2O \rightleftharpoons Zn(OH0_2 + H^{+} \qquad \log K = -15.29$$

$$2Zn^{2+} + H_2O \rightleftharpoons Zn_2(OH)^{3+} + H^{+} \qquad \log K = -8.80$$

Figure 2.9 Experimental complex formation curve of Z versus pL for the zinc(II)-caffeic acid system, at 25°C in 0.1 mol dm^{-3} (Na)[Cl], for the ligand to metal ratios as indicated.

Figure 2.10 Theoretical formation curves of Z versus pL using the zinc(II)-caffeic acid complexation constants given in Table 2.4 with identical titration conditions to those below.



The system of five complexes 'best' describing the data, as determined in this study, together with the refined values of the logarithms of their overall stability constants as well as those for some related ligands are given in Table 2.4.

The value for χ^2 is very large, as for all other zinc models, which seems a bit peculiar on scanning the residuals as the best scatter is observed with zinc amongst the three metals investigated.

A comparison with literature constants is possible only for similar ligands as the investigation of the zinc caffeic acid system zinc does not seem to have been reported before.

In this respect it is seen that the values of the constants for the 11-1 and 21-2 complexes are slightly higher than for the corresponding catechol species, by about 0.5 and 0.7 of a log unit respectively, using the values reported in reference (55). This again seems to indicate a possible influence by the carboxylic group on the chelating site.

Deciding on the best model for zinc(II) was very much easier than for copper. All other models with five or less complexes could be discarded on the basis of their R values alone, apart from not showing reasonable theoretical Zplots. The value of the Hamilton R factor pertaining to these experimental conditions at the 5% confidence level i.e. $R_{1000,500,0.05}$ is about 1.78. The next best alternative hypothesis, H_i having only four complexes gave an R_i/R_0 ratio of about 2.0 indicating the two systems to be statistically significantly different. One other system of five complexes which included the 32-3 polynuclear species had a compatible R value. The theoretical formation curve for this

Table 2.4

Logarithms of the overall stability constants, β_{pqr}, of zinc with caffeic acid, determined at 25°C and 0.1 mol dm^{-3} (Na)[Cl].

number of titrations : 9

pH range : 5.8 - 9.0

ligand concentration range : 0.0068 - 0.0275 mol dm^{-3}

corresponding $ZnCl_2$ conc. range : 0.0031 - 0.0027 mol dm^{-3}

pK_w used : 13.76

number of experimental observations : 458

χ^2 : 175.4

R : 0.002493

complex p q r	log β_{pqr}	SDEV in log β
1 1 0	2.99	0.056
1 1-1	-3.03	0.005
2 1-1	-0.39	0.034
2 1-2	-8.21	0.022
3 1-2	-5.51	0.017

Literature Data

Ligand	Complex p q r	log β_{pqr}	Medium (mol dm^{-3})	ref.
catechol	1 1-1	-3.52	1.0 KNO_3	52
	2 1-2	-8.60	"	"
	1 1-1	-3.30	0.1 KNO_3	55
	2 1-2	-8.90	"	"
	1 1-1	-3.52	*0.1 $NaClO_4$	54
	2 1-2	-8.88	"	"
3,4, dihydroxy-hydrocinnamic acid	1 1-1	-2.96	*0.1 $NaClO_4$	54
	2 1-2	-8.41	"	"

*temperature 30°C

model, whilst showing the correct trends was not nearly as superimposable with the set of experimental formation curves as was the pseudoplot of model H_0, given in Figure 2.10. These plots show even better overlap than was achieved in the copper caffeic acid system.

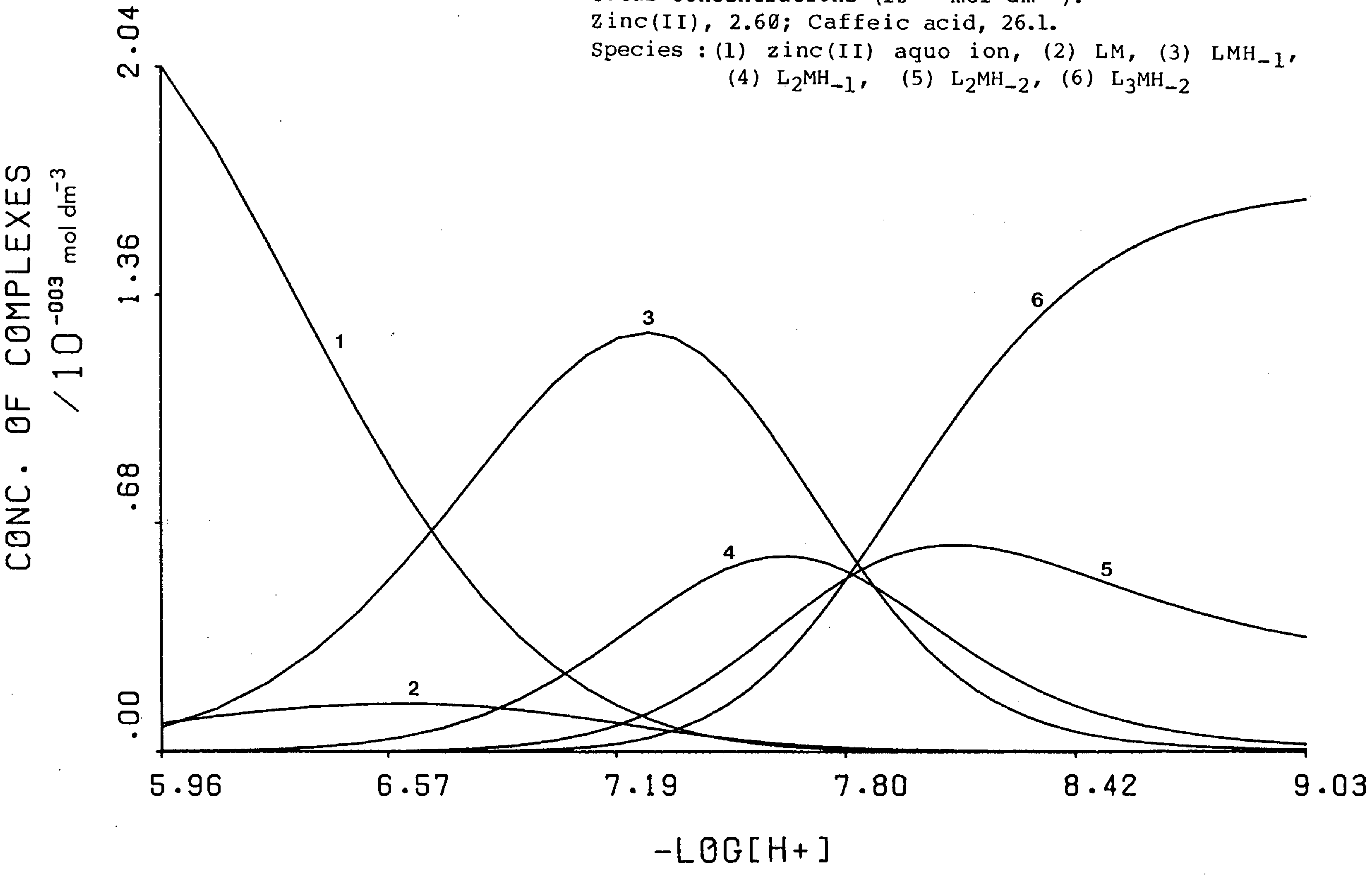
In contrast to copper, no polynuclear complexes were observed, however the tris complex 31-2 was formed as could possibly have been expected, due to the preference of zinc for 6 coordinate complexation. Unlike in the case with copper(II), the two bis complexes 21-1 and 21-2 are formed in the zinc caffeic acid system. However the 111 complex is absent, probably as the complexation takes place in the more basic region.

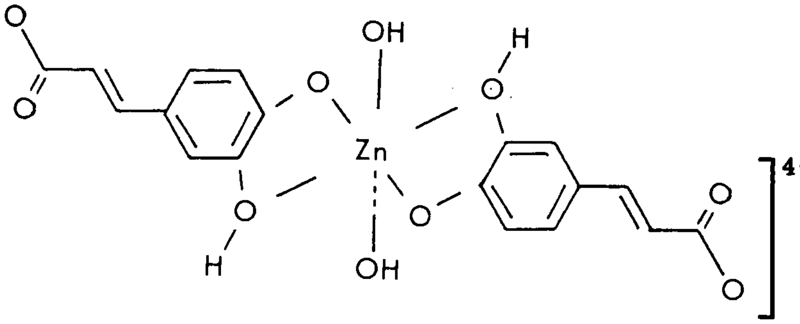
To predict the sites for complexation and thereby the actual structures, very similar arguments as in the case of copper were used.

Thus the proposed structures for the complexes 110 and 11-1 are thought to be identical to those for copper namely (d,e) and (f) respectively. As the 110 complex for zinc extends further into the basic region and is at the same time less significant, as can be seen by comparing the distribution diagrams Figures 2.8 and 2.11, the complexation probably has more of the carboxylic binding character, i.e. structure (e).

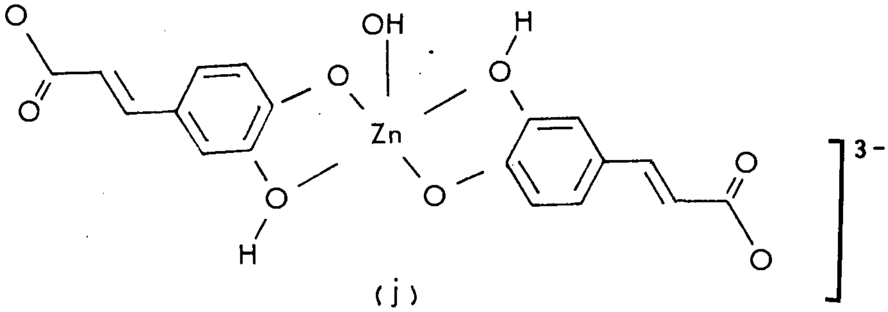
The 21r complexes are probably tetrahedral, although the 21-1 species could well exist as the five coordinate complex (j) which is not uncommon for zinc. Similarly the 21-2 structure could exist as the octahedral complex (k), with hydroxy ions occupying the other two co-ordination sites. However this is pure speculation as no evidence in favour of one or the other structure is available.

Figure 2.11 Distribution of species in the zinc(II) caffeic acid system.
Total concentrations (10^{-3} mol dm^{-3}):
Zinc(II), 2.60; Caffeic acid, 26.1.
Species : (1) zinc(II) aquo ion, (2) LM, (3) LMH_{-1},
(4) L_2MH_{-1}, (5) L_2MH_{-2}, (6) L_3MH_{-2}



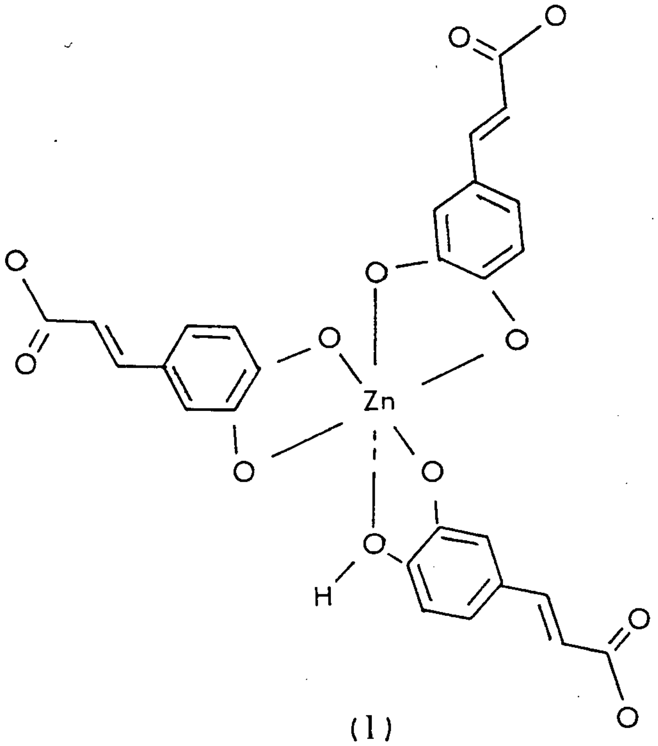


(k)

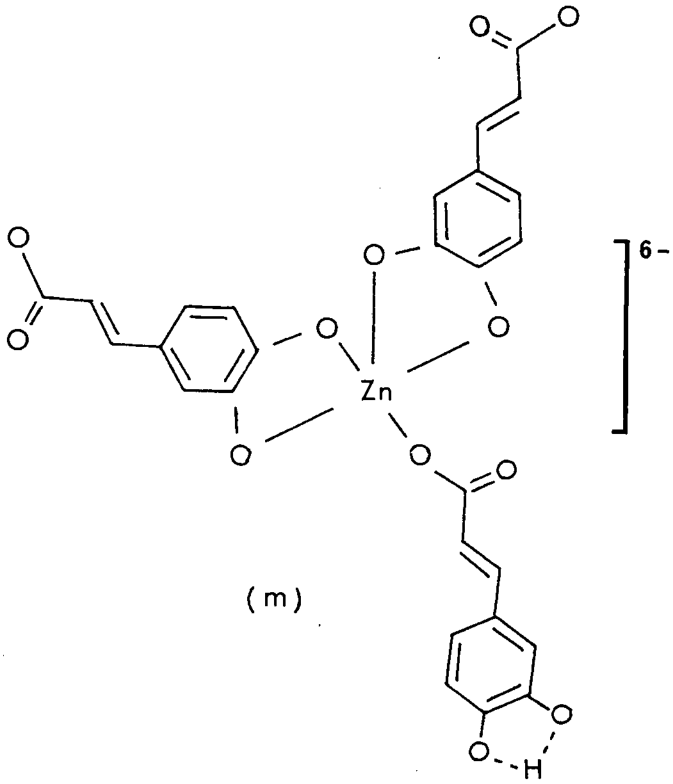


(j)

The tris complex 31-2 could possibly have the octahedral structure (l) or owing to stearic hindrance a five coordinate structure (m), which could account quite well for the one proton not lost on complexation.



(l)



(m)

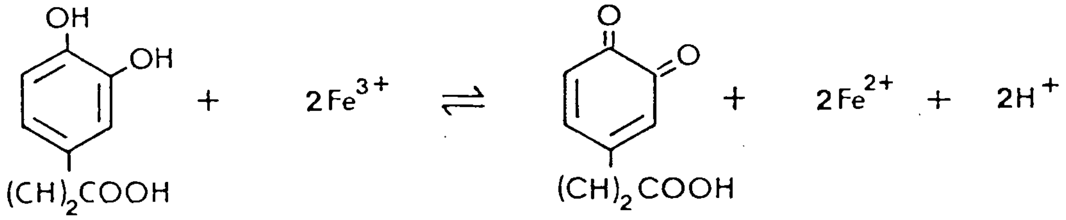
2.4.2.3 The iron(III) caffeic acid system.

On the addition of iron(III) chloride to acidic solutions of the ligand in ligand to metal ratios of 5:1 and 3:1 a distinct colour reaction was observed resulting in the formation of a greenly coloured solution which changed to dark blue black on titration with sodium hydroxide. This was so dark at about pH 5 that it was not possible to determine whether a precipate had formed or not. Although there was a definate reaction as indicated by the colour formation appearing at the sodium hydroxide inlet tip, very little change in EMF was observed during the 5 minute intervals between additions of titrant. To investigate the oxidation state of the iron during a titration small samples of the solution were tested at regular intervals with potassium thiocyanate and 1,10-phenanthroline. A control titration without caffeic acid was also run. This clearly showed that much of the iron(III) had been converted to the lower oxidation state even in the initial solution (about pH 1.7) before sodium hydroxide addition. During the course of the titration the iron(II) concentration was observed to increase as indicated by the qualitative measurement of the spot test and the iron(III) decreased to very nearly the detection limit, at a pH of about 8.

Indications of a slight brown precipitate were apparent at this point. The solid was considered to be $Fe(OH)_3$.

Owing to the increasing proportion of iron(III) substrate converted to iron(II) during a titration, the total analytical concentration of the reacting metal ion was unknown. Thus potentiometry could not be used directly as a method for investigating the complex formation between iron(III) and caffeic acid.

Hence it was decided to end the study of this system and proceed with the investigation of iron in the 2+ oxidation state. The result of the iron investigation so far can therefore only be used to indicate that iron(III), in an iron(III) chloride solution at least, is markedly reduced to iron(II) by caffeic acid. Considering only the respective potentials for the two half cell reactions this is not immediately obvious since the values are very similar. The EMF value of the full-cell reaction



is -0.022 volt, calculated from the value for the caffeic acid reduction potential of 0.792 volt (25). This seems to indicate oxidation of the iron(II) to iron(III). However, using the Nernst equation it can be shown that for a solution with initial concentrations of 1.0 and 0.5 mol dm^{-3} for caffeic acid and iron(III) respectively, i.e. simulating a 2:1 ligand to metal ratio titration at a pH equal to 1, about 90% and at pH of 2.84 about 99.8% of the iron(III) is reduced to iron(II). For simplicity no other interactions such as hydrolysis or complexation are considered.

2.4.2.4 The iron(II), caffeic acid system.

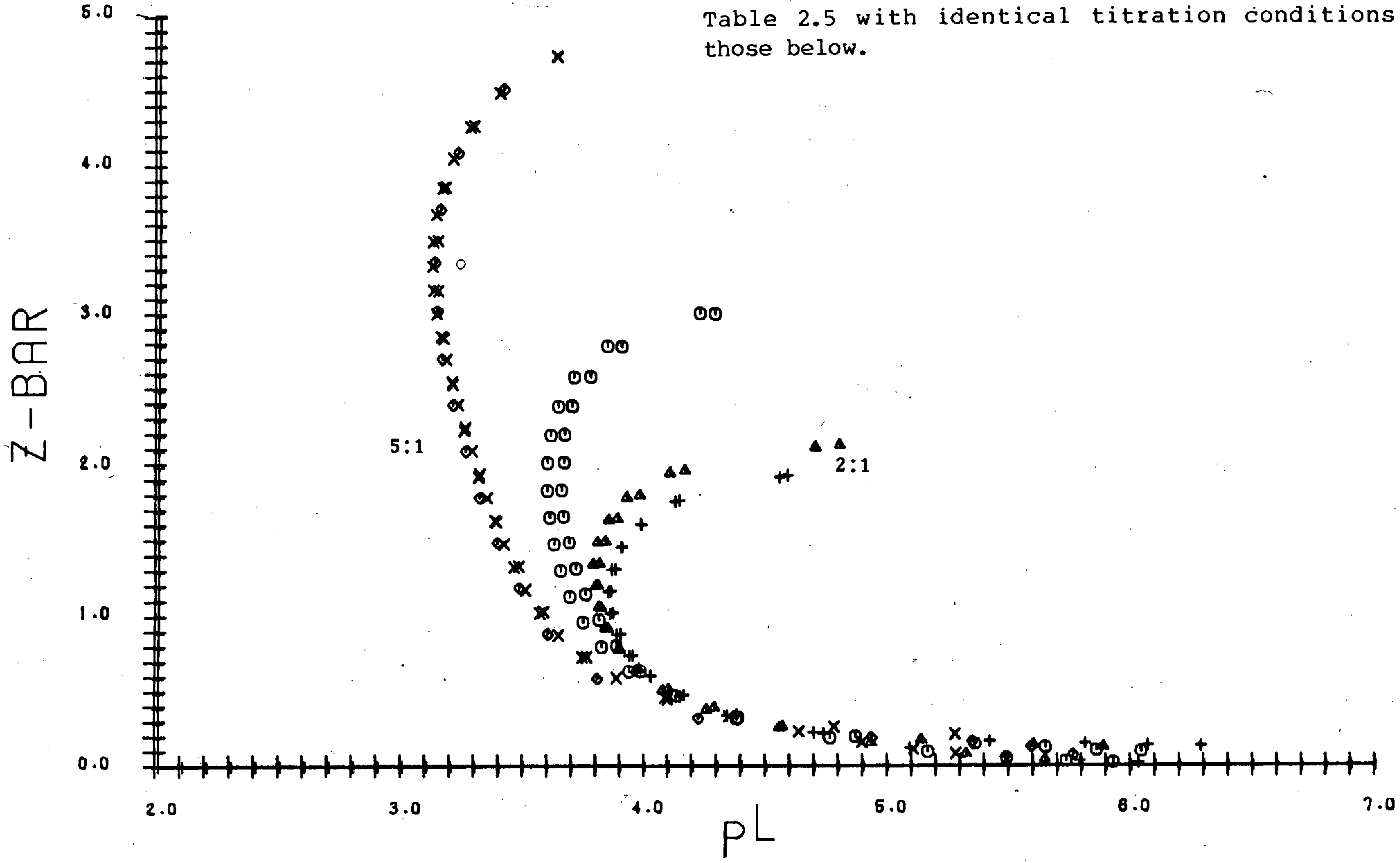
The last system to be experimentally investigated was that of iron(II) with caffeic acid. This was found to be the least reproducible system of those studied here. Williams et al attribute this to rapid deterioration of the iron(II) chloride solutions used in their studies with this metal ion (59). However the spot test with thiocyanate showed the iron(III) cocentration of the stock solution to be less than the detection limit of about 10^{-5} mol dm^{-3}, immediately prior to use in the determination. What probably caused this loss of precision was the fact that three different iron(II) chloride stock solutions with different total concentrations of protons and iron(II) were used in an effort to minimize systematic error and thereby increase the accuracy of the determinations (60).

Several replicated titrations were carried out over a smaller ligand to metal ratio range than for the other metals. The ratios were 5:1, 3:1, 2:1 and 1:1. As for the case with zinc, the 1:1 ratio titration showed precipitation and was therefore not included in the refinement procedure. A non reproducible 3:1 titration which had inadvertently been carried out too rapidly was also discarded. As can be seen from the experimental formation curves, Figure 2.12, the 2:1 ratio titrations show poor reproducibility. However they do not represent identical titration conditions as the total metal concentrations are not the same and therefore the split may be explained.

Similarly to before, the metal hydrolysis reactions were incorporated into every trial. These are given below together with the logarithms of their respective stability constants at 0.1 mol dm^{-3} ionic strength and 25°C (87).

Figure 2.12 Experimental complex formation curves of Z versus pL for the iron(II)-caffeic acid system, at 25°C in 0.1 mol dm^{-3} (Na)[Cl], for the ligand to metal ratios as indicated.

Figure 2.13 Theoretical formation curves of Z versus pL using the iron(II)-caffeic acid complexation constants given in Table 2.5 with identical titration conditions to those below.



$Fe^{2+} + H_2O \rightleftharpoons Fe(OH)^+ + H^+$ $\log K = -9.62$

$Fe^{2+} + 2H_2O \rightleftharpoons Fe(OH)_2 + 2H^+$ $\log K = -20.73$

$Fe^{2+} + 3H_2O \rightleftharpoons Fe(OH)_3^- + 3H^+$ $\log K = -31.89$

As was the case in the copper investigation it proved very difficult to decide on a single model which 'best' described the experimental data because several possible hypotheses were refined with insignificantly different R values.

Again most of these models could be rejected on the basis of non-superimposability of their formation curves with the set of experimental Zplots. This resulted in two most likely hypotheses with nearly indistinguishable Zplots, i.e. a 5 complex hypothesis H_0 given in Table 2.5 together with the logarithms of the overall stability constants, and a 4 complex model, H_i. For comparison Table 2.5 also includes literature data of the iron(II)-catechol system. As the R values for both models H_0 and H_i were about three times larger than for the other two metals studied, there is somewhat less certainty in the assignment of a best set of complexes. Both models are plausible on the basis of the chemical propagation principle. By applying Ockhams razor however, model H_0 may be rejected. However it was felt preferable to retain model H_0 as a tentative proposal for the following reasons. First, the formation curves were slightly better than those for H_i, especially in the pL region from 4 to 5. Secondly the one additional complex, namely 120, is relatively important as can be seen from the distribution diagram, Figure 2.14. As can be seen from Figure 2.13, apart from the one titration with a 3:1 ligand to metal ratio, the theoretical curves of hypothesis H_0, superimpose reasonably well with the

Figure 2.14 Distribution of species in the iron(II) caffeic acid system. Total concentration (10^{-3} mol dm^{-3}): Iron(II), 2.98; Caffeic acid, 14.9 Species: (1) iron(II) aquo ion, (2) LM, (3) LMH^{-1}, (4) L_2MH_{-1}, (5) L_3MH_{-2}, (6) LM_2

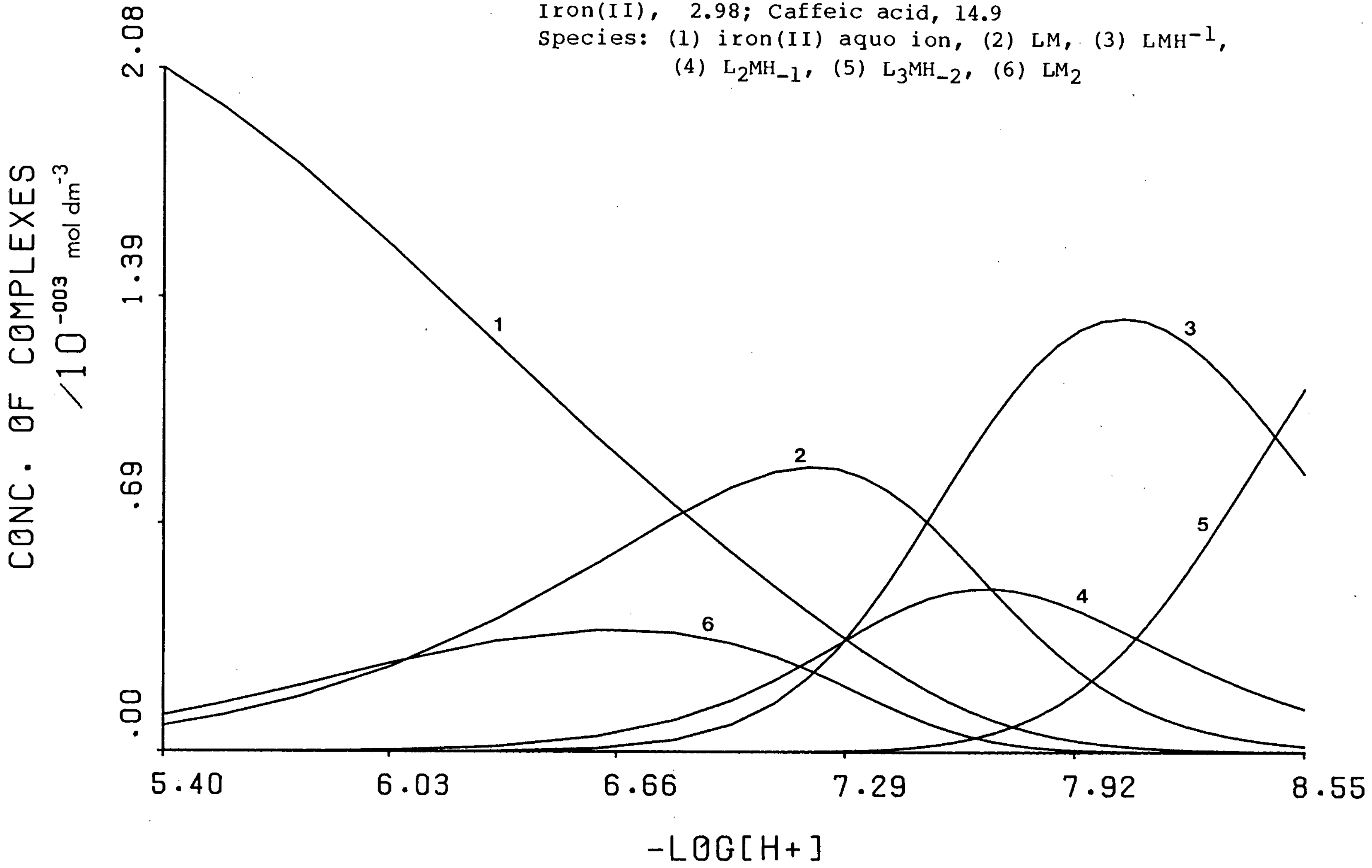


Table 2.5

Logarithms of the overall stability constants, β_{pqr}, of iron(II) with caffeic acid determined at 25^{o}C and 0.1 mol dm $^{-3}$ (Na)[Cl].

No. of titrations : 6

pH range : 4 to 8.5

Ligand conc. range : 0.0065 - 0.0150 mol dm $^{-3}$

corresponding $FeCl_2$ conc. range : 0.0032 - 0.0028 mol dm^{-3}

pK_w used : 13.76

no. of experimental observations : 262

χ^2 : 111.8

R : 0.007654

Complex p q r	log β_{pqr}	SDEV in log β
1 1 0	3.86	0.034
1 1-1	-3.83	0.054
2 1-1	-0.36	0.025
3 1-2	-6.14	0.023
1 2 0	6.69	0.093

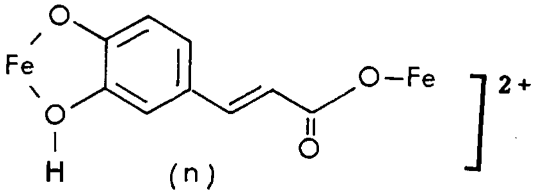
Literature Data

Ligand	Complex p q r	log β_{pqr}	Medium (mol dm^{-3})	ref.
catechol	1 1 0	3.52	1.0 KNO_3	52
	1 1-1	-5.10	"	"

experimental formation curves in Figure 2.12, even showing up the split observed in the two 2:1 ratio titrations. The latter lends some confidence to the constants proposed.

On removing the 3:1 titrations and one of the 5:1 from the data and refining over only 176 points, an R value for the modelled proposal of 0.0048 was obtained. The constants turned out to have nearly identical values although the standard deviations were lower. Comparing the values with those reported by Tyson and Martell (52) for the catechol-iron(II) system some differences are observed. These are probably due to more constants having been refined in our system, causing a certain amount of 'play off' between complexes, as well as the different background electrolyte conditions. No reasons are proposed why the differences in corresponding caffeic acid catechol constants are greater than observed for the other metals.

The complexes proposed of iron with caffeic acid are therefore quite similar to those of zinc, the only difference being the 120 species in place of the 21-2 complex. The actual structures of the complexes are proposed to be the same as those for zinc, except for the 21-1 and 31-2 complexes. These are unlikely to have the corresponding five co-ordinate structures proposed for the zinc complexes and are probably octahedral. The unique 120 complex, which is proposed with some reservation, is likely to have the following structure (n)



(n)

which is similar to that of the 12-1 complex of copper.

2.4.2.5 The manganese caffeic acid system.

As time did not permit the experimental determination of the stability constants of manganese (II) with caffeic acid, various constants were therefore estimated for this system, from those of copper(II) , zinc(II) and iron(II) with caffeic acid, determined in this study, by comparison with the literature constants of zinc(II) , copper(II) , iron(II) and manganese with catechol. In this way, taking the averages of the differences of corresponding catechol and caffeic acid constants for the iron, copper(II) and zinc(II) complexes and applying these to the manganese catechol constants, values for three manganese(II) caffeic acid complexes were obtained. These constants are given in Table 2.6 together with the complexes from which they were estimated. The value of 3.2 for the 110 complex is only very tentatively proposed, as this is based only on the constant β_{110} of the iron(II) caffeic acid system.

Based on the Irving-Williams series, manganese is expected to form similar complexes to those observed with iron(II) and zinc(II) with caffeic acid rather than those of copper(II) . Futhermore only constants corresponding to the equivalent catechol complexes were estimated.

The sites of complexation and therefore the probable structures are assumed to be the same as for those complexes of iron and zinc used to estimate the constants.

Thus the experimental determination of the various constants of the complexes of caffeic acid with copper(II), zinc(II) and iron(II) has been successfully concluded. However no constants could be obtained for iron(III) and those for manganese(II) were only estimated.

Table 2.6

Logarithms of the estimated constants β_{pqr} of manganese(II) with caffeic acid at 25°C and 0.1 mol dm^{-3} (Na)[Cl], together with the respective complexes of catechol and caffeic acid from which they were estimated.

Complex p q r	Estimated log β_{pqr}	Estimated from the constants of :
1 1 0	3.2	Fe, Mn -catechol Fe -caffeic acid
1 1-1	-5.0	Cu, Zn, Fe, Mn -catechol Cu, Zn, Fe -caffeic acid
2 1-2	-12.6	Zn, Mn -catechol Zn -caffeic acid

could be obtained for iron(III) and those for manganese(II) were only estimated.

CHAPTER THREE

MODELLING

3.1 Introduction

Nordstrom et al (61) define an aqueous chemical model as ' a theoretical construction which allows us to predict the thermodynamic properties of electrolyte solutions.' Most aqueous chemical models are based on the ion association theory of Bjerrum (61). Thus a more specific and complete definition of chemical modelling, applicable to this study, which indicates the various concepts involved is contained in the following quotation from Jenne (6) 'Chemical modelling encompasses the aqueous speciation of dissolved cationic elements among organic and inorganic ligands, of anionic elements among their complexes with cations, and both cationic and anionic elements among their redox states. Chemical modeling also includes calculation of the degree of saturation of an aqueous medium with regard both to metastable and equilibrium solids. ... Additionally , predictive chemical modeling must include kinetics.'

Two different techniques, which are however thermodynamically related, are currently used to solve the species distribution problem, i.e. of finding the most stable arrangement for a given solution. The first is the Gibbs free energy approach and the second is the so-called equilibrium constant approach. Both are subject to the conditions of mass-balance and chemical equilibrium. The Gibbs free energy approach, first proposed by Dantzig and co-workers (62), involves minimizing the free energy function

$$\Delta G_m^{\ominus} = -RT\ln K$$

for a given set of species, subject to the restraints of the mass-balance requirements. The equilibrium constant approach, proposed by Brinkley (63), is probably used by the majority of workers in this field of study and has many prominent applications. In this case, the solution to the problem is obtained by substitution of the mass action expressions into the mass balance equations. This results in a set of non-linear simultaneous equations, which are usually solved by an iterative procedure as the exact solution is not feasible. Details are discussed in section 3.2. The different mathematical techniques used in the two approaches have been comprehensively reviewed by Zeleznik and Gordon (64). It has been suggested that faor large, complex systems the equilibrium constant approach is preferable since there are at present more reliable and available equilibrium constants than free energy values. Accordingly it was decided to choose the equilibrium constant approach for the current study involving plant nutrient solutions.

The classical example of a multicomponent equilibrium study is probably that of Sillen and co-workers on the speciation of seawater (65). Since then the speciation in a great number of widely diverse systems has been investigated by the use of various chemical models. As an indication of the diversity of the systems that have been succesfully studied , a few specific examples are given here. These are, the speciation of trace elements in natural waters (66), blood plasma models (67), acid mine drainage (61), zinc uptake in the human digestive system (68), complexation in xylem sap (69), a soil solution model (70) and the speciation in urine (74).

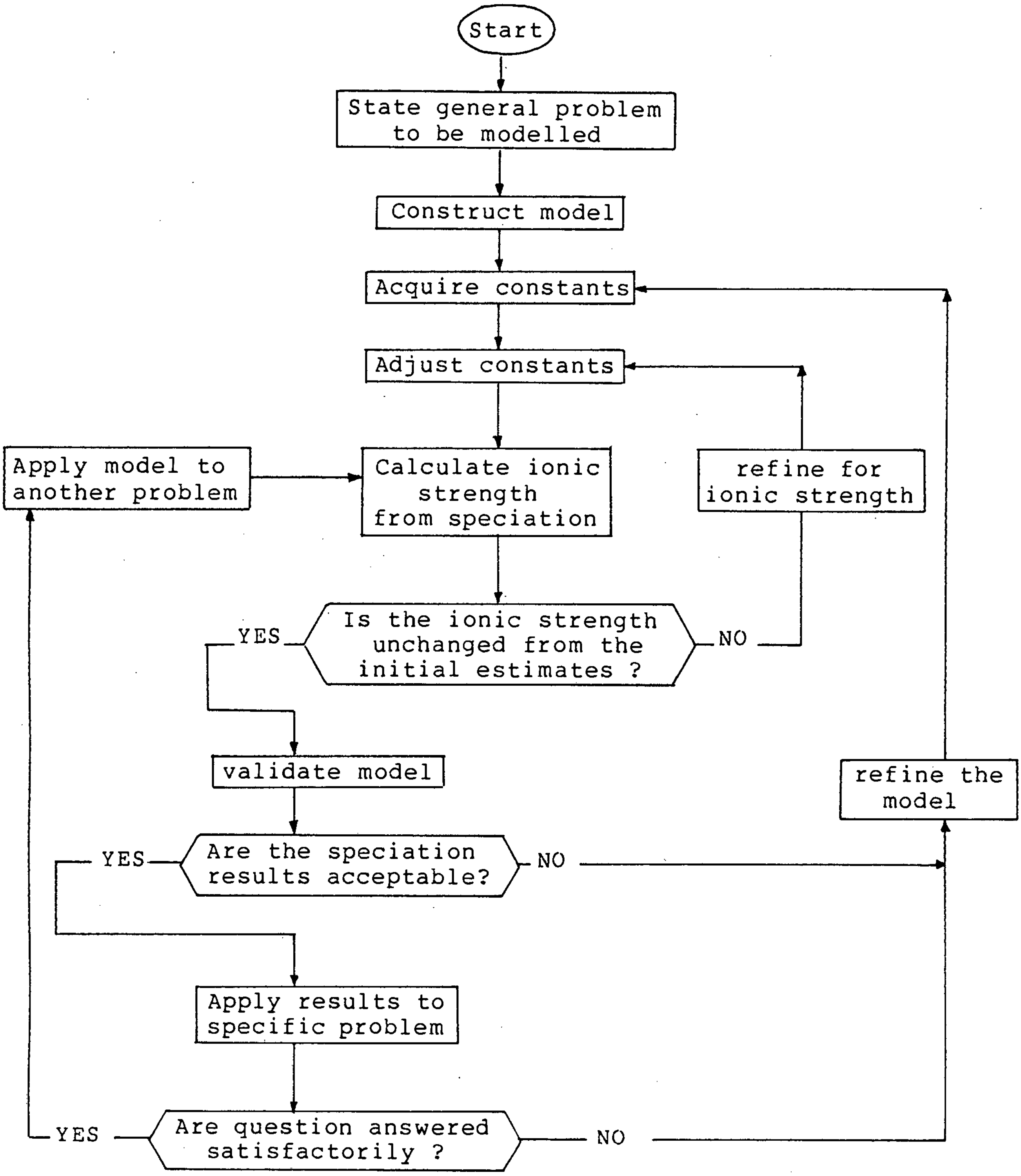
Various computer programs have been developed over the years to solve the mathematical calculations involved in the modelling of the chemical equilibria. These differ from one another by the numerical techniques used and by incorporation of additional features in some cases. The choice of a particular program is dependent upon the requirements which may vary from one study to another. The criteria arising from the nutrient solution model and specified in this study are the following. The program chosen should be able to handle a great many soluble species and several redox equilibria as well as a large number of solids. It should also incorporate some form of ionic strength adjustment of the equilibrium constants. Of the speciation programs available in this laboratory, i.e. COMICS (71), ECCLES (72), MINEQL (73), REDEQL2 (75) and PSEUDOPLOT6 (70) (which is a modified version of HALTAFALL (4)), the second generation programs of the REDEQL family were an obvious choice. These are based on the use of the Newton-Raphson iterative convergence procedure. Of the two programs MINEQL is the more compact with greater clarity and flexibility and was thus chosen for the work in this study. The additional features of the much more elaborate program REDEQL2 were not required here.

Having chosen the particular modelling approach and a suitable program the aim was then to construct a reliable nutrient solution model to study some of the factors affecting the uptake of nutrients by plants. At this stage some of the problems that are encountered in most modelling studies should be pointed out. These are an inadequate knowledge of natural organic ligands, of redox processes, of thermodynamic, time-dependent and kinetic information and of anthropogenic input. Added to this is the availability of and uncertainty in much of the thermodynamic data especially for many of the solids. These problems require various assumptions to be made, (see sections 3.2 and 3.3), which are critical to the study. This is because any model can thus only be as good as the assumptions on which it is based.

As modelling largely involves computational procedures so can the various processes involved in a modelling study be presented by a flow diagram. Such a diagram is presented in figure 3.1 which indicates the proposed plan of approach of investigation, to be used in this particular study.

To end off we would just like to make the following pertinent observation with regard to the philosophical approach to modelling. Morel and Morgan (76) state that equilibrium models can be very useful for understanding the various processes - dissolution and precipitation, oxidation and reduction, acid base and coordinative interactions which govern the chemical composition of natural waters. It is crucial to bear the above in mind as it illustrates the finer distinctions that should be made in the approach to modelling in general. All too often computer based models are judged on their ability to represent reality (77). This should however not be the fundamental aim of a modelling study and if it were indeed to achieve this, it would by definition no longer be a model. A model should rather be used as an analytical technique to help understand the processes involved in the system studied.

Figure 3.1 Flow diagram showing the processes in a chemical modelling study.



3.2 Theory

The general theory of chemical modelling of the speciation in solution has been dealt with in the literature quite extensively (6, 62, 63). Hence only a broad outline of the principles involved and the assumptions made will be given here. A few definitions of importance to the following discussion of chemical equilibria are given first.

The chemical potential of a species A, in solution, can be defined by the equation

$$\mu_A = \mu_A^{\ominus} + RT\ln a_A \qquad \ldots 3.1$$

where $\mu_A^{\ominus}$ is the potential of A in the standard state and
a_A is the activity of species A in solution.
R is the gas constant equal to 8.314 $JK^{-1}mol^{-1}$ and
T is the temperature in Kelvin.

For the general reaction

$$A + B \rightleftharpoons C$$

the following relationship is then applicable at equilibrium

$$\mu_A + \mu_B = \mu_C$$

The equilibrium constant, K, for the above reaction is defined by the equation

$$K = a_C / (a_A x a_B)$$

Using equation 3.1 the following important relationship is then obtained

$$\Delta G_m^{\ominus} = - RT\ln K \qquad ...3.2$$

where $\Delta G_m^{\ominus}$ is the standard molar Gibbs free energy for the above reaction, and is given by

$$\Delta G_m^{\ominus} = \mu_C^{\ominus} - \mu_A^{\ominus} - \mu_B^{\ominus}$$

As a starting point in any modelling study the components of the proposed chemical model must be chosen, based on the composition of the aqueous solution to be investigated. This is then followed by the process of determining all the possible complexes that could potentially be formed from these components. The next stage consists of acquiring values for the respective equilibrium constants and solubility products. (Many equilibria may be disregarded if their constants are insignificant.) Most of these constants may be obtained from the literature. Where a paucity of data exists in the literature, these constants are required to be experimentally determined or otherwise estimated from the values for similar systems. The latter approach is often not very satisfactory if any quantitative determinations are to be made.

In some cases equilibrium constants are not directly available but values for the standard molar enthalpy and entropy of the species involved in a reaction are known. Rearranging equation 3.2 above, the lnK of the reaction can be calculated from the following

$$\ln K = - \Delta G_m^{\ominus} / RT \qquad ...3.3$$

where $\Delta G_m^{\ominus}$ is obtained from the relationship

$$\Delta G_m^{\ominus} = \Delta H_m^{\ominus} - T \Delta S_m^{\ominus} \qquad \ldots 3.4$$

Redox equilibria are mostly reported in the form of thermodynamic reduction potentials for the relevant redox half-cell reactions. These are related to the equilibrium constants, K, by the following reaction

$$EMF = (^{RT} / _{nF}) \ln K \qquad \ldots 3.5$$

where EMF is the standard hydrogen redox potential given in volts

R, T and F are defined as for equation 2.10 and

n is the no. of moles of electrons involved in the reaction.

Furthermore, the measured reduction potential of solution, i.e. Eh can be related to the electron activity by the equation

$$Eh = \frac{2.303 \; RT}{F} \, pe \qquad \ldots 3.6$$

where Eh is the voltage difference between the platinum and the standard hydrogen electrode, and

pe is the negative logarithm of the electron activity.

Having obtained the stability constants many of these may require temperature and ionic strength adjustments, to the conditions of the model as specified in the study. Temperature corrections are made using the Gibbs-Helmholtz equation applied to chemical reactions at constant pressure, P.

$$\left(\frac{(\partial G/T)}{\partial T}\right)_P = {}^{-\Delta H} / _{T^2} \qquad \ldots 3.7$$

This gives on substitution of equation 3.2 the van't Hoff equation

$$\left(\frac{\partial(\ln K)}{\partial T}\right)_P = \Delta H_m^{\ominus}/_{RT^2} \qquad \ldots 3.8$$

where $\Delta H_m^{\ominus}$ is the standard molar enthalpy of reaction and is dependent on temperature

On making the assumption of constant $\Delta H_m^{\ominus}$ over the small temperature intervals used in this study, the following approximate relationship is obtained by integrating equation 3.8

$$\ln\left({}^{K_2}/K_1\right) = \left({}^{H_m^{\ominus}}/R\right)\left({}^{1}/T_1 - {}^{1}/T_2\right) \qquad \ldots 3.9$$

The variation of formation constants with changes in ionic strength has been studied by Linder and Murray (28). The following extended Debye-Hückel equation was used as the basis for the extrapolation of stability constants to different ionic strengths

$$-\log \gamma_i = \frac{A\, z_i^{\,2}\,(1 + I^{1/2})}{1 + B\mathring{a}_i I^{1/2}} + C \times I \qquad \ldots 3.10$$

where γ_i is the activity coefficient of the i^{th} species defined by equation 2.2

A and B are constants characteristic of a particular solvent and temperature (for water at 25°C, A=0.509 and B=0.328)

I is the ionic strength given by equation 2.5

$\mathring{a}_i$ is an ionic size parameter given in Ångstrom

C is an empirical constant

z_i is the charge of the i^{th} ion

This makes the assumption that ionic strength does not affect the activity of uncharged species.

Essentially, Linder and Murray used the fact that for a particular complexation reaction of two components, for example between a metal ion and a ligand

$$pL + qM \rightleftharpoons L_pM_q$$

the equilibrium constants K_1 and K_2 at ionic strengths I_1 and I_2 are related by

$$K_2 = K_1 \times Q_1 / Q_2$$

where each Q is an activity coefficient quotient defined similarly to equation 2.4 but only for two reactants in this case.

These activity coefficients and thereby the quotients can be calculated using equation 3.9 provided that values for the constants $\overset{o}{a}_i$ and C are available.

Kielland (78) and Truesdel and Jones (79) have listed a_i values for a wide range of ions. Making the assumption that C has the same value for each ion in a given complexation equilibrium Linder and Murray obtained an expression that can be used to calculate C values from two equilibrium constants at different ionic strengths. This overall C value can then be used to predict the equilibrium constant at the required ionic strength. Where equilibrium constants were only available at one ionic strength C values where estimated from related equilibria or the generally recommended value of -0.1 was used. It was shown that the accuracy obtained using this approach for adjusting equilibrium constants to 0.04 mol dm^{-3} ionic strength as required by the soil model was sufficient for the purposes of the modelling study (28).

Most of the nutrient solutions modelled in this study, section 3.5, have an ionic strength of 0.015 mol dm^{-3} or less. As the above procedure was intended for use at low ionic strengths it was decided to use this approach in the present study. For the purposes of the calculations required, the Fortran computer program, LOGK, developed by Murray (70) was used here.

An analagous procedure to the above was developed for the adjustment of solubility products, in this study. Time did however not permit the testing of this procedure as thoroughly as desired. However, it is generally suggested that reported constants are a greater source of error than the ionic strength adjustments made, especially when these are for only small differences (28, 6). Furthermore, the approach used here is based on very similar assumptions to those made in the study by Linder and Murray, as well as making use of similar mathematical equations and computational procedures. This lends some confidence to its use for solubility products. Added to this is the possibility that the assumption of a unique C value for all the species in a particular reaction is more valid in this case. This is because there are necessarily less charged species for which constant C is assumed in a solubility product than in a complexation equilibrium due to the uncharged solid. Therefore this untried procedure was used with some confidence for the adjustment of solubility products to different ionic strengths. The computational calculations were carried out using the Fortran program LOGKSP, developed in this study.

The rest of this section on theory is involved with the mathematical solution of the speciation problem at chemical equilibrium. This is specific to the equilibrium constant approach and particular to the computer program MINEQL. The concentration of the complex in chemical equilibrium with its components can be expressed as a function of the specific component concentrations by the mass action equation. This is illustrated by the following simple example for the metal ligand complex, ML_2

$$[ML_2] = \beta_{ML} \times [M][L]^2$$

In general, the above equation for all complexes in the solution using the notation of reference (73), can be represented by

$$C_i = \beta_i \prod_{j=1}^{n} X_j^{a(i,j)} \qquad \ldots 3.11$$

for i=1 to m

where C_i is the concentration of complex i

β_i is the stability constant of complex i

X_j is the concentration of component j

a(i,j) is the stoicheiometric coefficient of component j in complex i

The problem is thus essentially reduced to finding the set of free concentrations of all the components in the solution that will satisfy the mass balance conditions. This condition states that the free concentration of component j plus the amount of j in all of the complexes should be equal to the total concentration of j, (T_j). An example of a mass balance equation for the ligand species in a system of three components is given in section 2.2 by equation 2.9.

This can be rearranged to give the following relationship in the general case with i complexes and j components

$$Y_j = T_j - \sum_{i=1}^{m} a(i,j)\ C_i \qquad \ldots 3.12$$

where Y_j is the difference between the total imposed analytical concentration of component j (T_j) and the amount of component j in all the complexes C_i as computed by equation 3.11

T_j is the total analytical concentration of component j

The exact solution to the problem is thus the set

$$\{ X_j : j=1,n \}$$

such that all Y_j's = 0. To solve the problem MINEQL calculates the concentration of each complex C_i using initial estimates of the free component concentrations X_j. Following this the total concentrations of all the components j are calculated, i.e. the second term in 3.12. Then by iterative use of the Newton-Raphson method, the difference functions, i.e. equation 3.12, and their derivatives are used to find improved values for the X_j's. These are resubstituted into equation 3.11 and the process repeated such that the Y_j's are minimized.

In practise the roots of the equation Y(X) = 0 are only approximately determined. Thus a convergence criterion needs to be established, that when each Y_j becomes sufficiently close to zero the system is taken as solved. As each Y_j is the sum of terms that vary greatly in order of magnitude, the convergence criterion used in MINEQL is chosen to reflect the magnitude of Y_j relative to the maximum of the terms of which Y_j is the sum. Thus, the criterion for convergence is given by

$$\frac{|Y_j|}{\max(Y_j)} < \epsilon \qquad \ldots 3.13$$

for all $j = 1,n$

where $\max(Y_j)$ is the maximum value of the terms in equation 3.12 and

ϵ is a small positive user specified number

In order to avoid matrix inversion, the iterative formula obtained from the Newton-Raphson method to solve the roots of the equation $Y(X) = 0$ is modified and the resulting Jacobian is solved by Gaussian elimination. Futhermore, to improve the computational efficiency, MINEQL makes use of a transformation of basis. This has the effect that the Jacobian approaches a diagonal matrix, which leads to faster solution of the problem.

Once convergence has been obtained MINEQL then checks the solubility of all the potential solid phases. If it occurs that the calculated free concentration product exceeds the solubility product, precipitation of that particular solid is indicated. This effectively changes the total analytical concentrations of the involved components. Thus the problem has to be modified and, as before precipitation, is again iteratively solved until convergence is obtained. Any precipitated solid can, of course, dissolve, resulting in an effective increase of the free concentrations of the involved components. This again requires the problem to be solved as before. Thus only when no further solids dissolve or precipitate and the convergence criterion is satisfied, is the computation of the equilibrium set of aqueous species and solids taken as completed.

3.3 Model Construction

3.3.1 Selection of components

Many different nutrient solutions of various compositions with considerably different concentrations have been used over the past years in a large number of plant studies (80). The differences in composition are largely due to the particular salts that were used in making up the solutions and due to the requirements of particular effects studied. The greatest variation of components noticed in the literature was for the synthetic ligands used to complex iron. However, what most of these solutions have in common are the nutrients essential for plant growth, which have only quite recently been established. These can roughly be divided into macro- and micronutrients on the basis of the actual concentrations required. Thus a set of components exceeding 20 in number was built up as being reasonably representative of the experimental solutions in current use by researchers in the field of plant nutrition, and are listed in Table 3.1. These therefore, form the basis of the plant nutrient solution model, giving rise to the various aqueous equilibria and potential solids, as proposed in this study.

As can be seen from Table 3.1 the electron is taken into account here as well, as it is required for the purposes of the redox reactions. Furthermore the electron activity can also be used as a measure of the reduction potential of a solution. Usually redox potentials of solutions are reported in terms of Eh values. Sillen pointed out however that the electron can be considered as any other reactant or product in chemical reactions (82). This lead to the theoretical concept of 'free electrons' in solution (83) which are related by their activity to the Eh by means of equation 3.5. This concept of 'free electrons' in solution, is thus much more useful than that of Eh in equilibrium studies, as now all the equilibria can be written in terms of concentration.

Protons on the other hand are considered here as arising from the dissociable hydrogen ions of protonated ligands and do therefore have input concentrations. Hydroxy ions are taken into account implicitly by including the ionic product of water amongst the equilibrium constants of the model and need therefore not be listed as a separate ligand. The different redox states of some metals are considered and are therefore also listed as components, although usually the particular micronutrient is only input in one form. The model was also considered to be in gaseous equilibrium with the atmosphere. Oxygen and carbon dioxide are however involved by the use of conditional equilibrium reactions and are thus not input as components as such. This is further explained in the following section 3.3.2.

As has already been mentioned above, the actual concentrations of these components vary greatly from one published solution recipe to another. Therefore the particular concentrations and components used in the present work (always a subset of those given in Table 3.1) are presented separately for each solution modelled in section 3.5

Table 3.1

Components of the nutrient solution model used in this study.

Cations	Ligands
calcium	carbonate
magnesium	sulphate
potassium	*chloride
#sodium	ammonia
*iron(III)	nitrate
*iron(II)	phosphate
*manganese(III)	#citrate
*manganese(II)	#[a]EDTA
*copper(II)	*borate
*copper(I)	*molybdate
*zinc(II)	#[b]DTPA
protons	#[c]EDDHA
	#caffeate
	electrons

* Micronutrients

\# non-essential elements for plant growth as reported in reference (81)

[a]EDTA = ethylenediaminetetraacetic acid

[b]DTPA = ethylenetriaminepentaacetic acid

[c]EDDHA = ethylenediaminedi(o-hydroxyphenylacetic acid)

3.3.2 Acquisition of stability constants and solubility products.

For most of the roughly 260 equilibria arising from the components listed in Table 3.1 the formation constants were obtained from the invaluable collections of stability constants by Högfeldt and Perrin (85, 86), Sillén and Martell (82, 84) and Martell and Smith (87, 88). Where more than one value for a particular equilibrium constant existed the choice of a 'best' value was based on the following criteria :

the method of determination;
the journal of publication;
the workers who determined the constants;
the conditions such as temperature, ionic strength and background electrolyte;
and being representative of the values for the constant reported by other workers.

Only seldom was it required to calculate constants from thermodynamic data. Usually therefore, this method was used only as a check on the equilibrium constants reported.

Various solids were found to have a wide range of reported solubility products. As these solids can have a profound effect on the composition of the solution if they are found to precipitate, see section 3.2, accurate constants are therefore of considerable importance. In fact only the solids that precipitate or are close to precipitation as determined by their solubility products and the conditions of the solution need be considered. Amongst others these are the following: calcium hydroxy apatite, various iron hydroxy solids, iron phosphate and calcium carbonate. Some of these are also expected to have a direct effect on the pH by involvement of the hydroxy species. It is therefore felt that some discussion of the values used in this model for the more important constants including those of the two atmospheric equilibria, is required here.

3.3.2.1 The iron hydroxy solubility products.

The choice of solubility products of iron with hydroxy ions is rather difficult as there exist wide ranges of values for the important solids.

Considering the amorphous $Fe(OH)_3$ solid a range of values for logK from -4.9 (79) to -2.5 determined by Schindler in 1963 (89) is reported for the reaction

$$Fe^{3+} + 3H_2O \rightleftharpoons Fe(OH)_3(s) + 3H^+ \qquad ...3.14$$

at 0.0 mol dm^{-3} ionic strength, depending on whether the study was on freshly precipitated or aged amorphous solid. Biedermann and Schindler (90), studying the steady state obtained after 200 hours, report a value of logK = -3.96 for amorphous ferric hydroxide in 3.0 mol dm^{-3} sodium perchlorate which adjusts to -2.98 at 0.0 mol dm^{-3} ionic strength. For the more stable solid FeO(OH) known as geothite, Schindler (89) reports a value of log K = -1.4 in 3.0 mol dm^{-3} sodium perchlorate from studies of solutions which were aged for several years. This corrected for ionic strength, adjusts to give a value of -0.5 at infinite dilution. As the modelling study is based on the assumption of thermodynamic equilibrium the problem exists of which form of the solid to chose, let alone which stability constant value to use, for the 'shorter term ' nutrient solutions studied here.

Lindsay (83) in the study of soil solutions uses a value for logK of -3.54 for the reaction 3.14. This is calculated from the thermodynamic data of Schindler reported in 1963. Nordstrom et al in their investigations using WATEQ2 (61) use a value for logK of -4.90 obtained from the studies of Langmuir and Wittemore for the same reaction. Furthermore they claim that their experimental measurements of spring waters as well as those of other workers fall well within a range for logK of -5.0 to -3.0.

Therefore as a representative value, that reported in reference (88) for the logarithm of the solubility product of $Fe(OH)_3$ of 38.8 was chosen. From this a value for logK of -3.20 was calculated for the reaction 3.14 above, which corresponds well with the value of Platford (85) of -3.40.

Similar problems were encountered with the solid magnetite formed by the following reaction

$$3Fe^{3+} + e^- + 4H_2O \rightleftharpoons Fe_3O_4(s) + 8H^+ \qquad ...3.15$$

which is believed to exist in saturated solutions with increased reduction potentials. This has an even larger range of solubility products than the other iron oxides. Truesdel and Jones (79) proposed a value for the logarithm of the constant for reaction 3.15 above, of 9.57, calculated from values reported by Nriago in 1972. In contrast Lindsay (83) reports a value of 3.42 calculated for the same reaction 3.15 from the thermodynamic data of Larson et al 1968, Salmon 1961 and Gronvold and Westrun 1959, which is 6 log units different to the above value of Truesdel and Jones! Lindsay in the same reference (83) reports a value of -35.69 for the log K_{sp} of the same solid but formed by the different reaction 3.16, given below.

$$3Fe^{2+} \rightleftharpoons Fe_3O_4 + 8H^+ + 2e^- \qquad ...3.16$$

Using a logK of 13.02 for the iron redox half cell reaction a value of logK = 3.37 for the reaction 3.15 is obtained by mathematical manipulation. This compares quite favourably with the other value quoted by Lindsay (83). Ball et al (91), using the program WATEQ2, therefore probably use the associated data base obtained from the work by Truesdel and Jones. They do however state that they prefer the thermodynamic data of Larson and Hepler 1968 especially for the iron(II) species over that reported by the National Bureau of Standards (49). Thus it is assumed that they also use the lower value of 3.42 as do Lindsay and co-workers, which is therefore used in the present study. This results in a value of 9.60 for the constant of the reaction below as required in the input to MINEQL.

$$2Fe^{3+} + Fe^{2+} + 4H_2O \rightleftharpoons Fe_3O_4 + 8H^+ \qquad ...3.17$$

Other studies report values for magnetite but only for conditions of elevated temperatures and pressures and are therefore of little use here.

The last iron oxide that will be discussed here is ferrosic hydroxide. This is proposed to be a metastable precursor, transforming into magnetite or iron hydroxide on the ageing of saturated solutions (92). A value of logK = -17.67 was chosen in this study for the constant at an ionic strength of 0.0 mol dm^{-3} and 25^{o}C for the formation of ferrosic hydroxide by the following reaction

$$2Fe^{3+} + Fe^{2+} + 8H_2O \rightleftharpoons Fe_3(OH)_8 + 8H^+ \qquad ...3.18$$

This was calculated from the value of logK = -43.75 (83) for the reaction

$$3Fe^{2+} \rightleftharpoons Fe_3(OH)_8 + 8H^+ + 2e^- \qquad ...3.19$$

A value of logK = -16.84 is calculated from the results of

Ponamperuma et al (93) for reaction 3.18. Both values are however dependent on the iron(III) hydroxide constant and are thus subject to similar errors, see above. Thus Ball et al (91) using the data of Ponamperuma et al calculate a value for logK of -20.22 for reaction 3.18 above. The value used in this study is based on that of the iron hydroxide constants of logK = -3.20 and seems relatively reasonable.

The model will however only consider this solid for potential precipitation if the two equilibria 3.14 and 3.17 representing the thermodynamically more stable solids, ferric hydroxide and magnetite, are absent.

3.3.2.2 The calcium hydroxy apatite constant.

The next solubility product investigated was that of calcium hydroxy apatite which can be described by the following reaction

$$Ca_5OH(PO_4)_3\ (s) \rightleftharpoons 5Ca^{2+} + OH^- + 3PO_4{}^{3-} \qquad ...3.20$$

This solid was found to precipitate quite frequently in the solutions modelled in this study and can therefore greatly influence the solution composition, for the reasons given in section 3.2. Futhermore, this was found to have one of the largest ranges of all the solids included in the model and a representative value for its constant is therefore of considerable importance.

The components involved are relatively important in their own right. For example phosphate forms the quite stable solid, $FePO_4(s)$ which in turn could thus affect the iron concentration, as well as having a direct influence on the free hydrogen ion concentration by the various hydrogen phosphate ion pairs.

Equally the hydroxy ion is obviously of some importance to the computed pH of the solutions. Thus a short review of the literature on the determination of hydroxy apatite solubility products was undertaken.

In the article by Madsen (94) the following main reason for the difficulty of determining the solubility product of hydroxy apatite was advanced. This is namely due to the approach from either supersaturation or undersaturation in the various experimental studies. Furthermore, various other difficulties exist in the determinations. Bjerrum is quoted (94) as reporting a range of values from -56.7 to -57.7 for the logarithm of the solubility product, i.e. log K_{sp} for reaction 3.20, due to point defects. Moreno et al on the other hand are quoted as reporting a range for logK_{sp} from -54.6 to - 57.4 due to different sample treatments. Wier, Chien, and Black (95) give a range for the log K_{sp} of -58.2 to -60.25, representing the lowest values ever determined. They propose the difference to be due to hydroxy apatite of different activities resulting in deviations therefore, on using different total amounts of solid in a fixed solution volume.

Verbeek et al (96) give values for 5 differently prepared calcium hydroxy apatite samples. 4 of these are quite consistent giving a range of best estimates for the thermodynamic log K_{sp} of -57.48 to -58.54. Much care seems to have been taken in this investigation and the determinations of other workers are also discussed. Thus values falling within this range,x are thought to represent suitably accurate estimates of some 'real' value. Clarke (97) reports a value of logK = -57.75 which falls well within this 'best' range of Verbeek et al, and is therefore used in this study. This results in a value of log K = 43.75 at 0.0 mol dm^{-3} ionic strength and 25°C for the reaction

$$5Ca^{2+} + 3PO_4^{3-} + H_2O \rightleftharpoons Ca_5OH(PO_4)_3 + H^+ \qquad ...3.21$$

as required for the input to MINEQL.

3.3.2.3 The carbonate equilibria.

It has been assumed in this study that the model is in equilibrium with atmospheric carbon dioxide which can be described by the reaction

$$2H^+ + CO_3^{2-} \rightleftharpoons H_2O + CO_2(g) \qquad ...3.22$$

A value for the logarithm of the equilibrium constant for reaction 3.22 can be calculated in the following way. From the reactions

$$H^+ + CO_3^{2-} \rightleftharpoons HCO_3^-$$

with logK = 10.330 (98), and

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3^*$$

with logK = 6.351 (99), a value for the reaction

$$2H^+ + CO_3^{2-} \rightleftharpoons H_2CO_3^* \qquad ...3.23$$

of logK = 16.68 at 25°C and 0.0 mol dm^{-3} ionic strength is calculated, where $H_2CO_3^* \equiv H_2CO_3^o + CO_2(aq)$ by convention, with $CO_2(aq)$ / $H_2CO_3^o$ in a ratio of about 650:1 (101) and $H_2CO_3^o$ is the uncharged ion pair.

A value of logK = -1.466 (99) for the solubility of gaseous carbon dioxide in water, described by the reaction

$$CO_{2(g)} + H_2O \rightleftharpoons H_2CO_3^*$$

was used, which takes the vapour pressure of water into account. This compares well with that of Helgeson of -1.465 (88). Thus using this and reaction 3.23 a value of log $K_{(g)}$ = 18.146 could be calculated for reaction 3.22.

This gives at a fixed partial pressure for carbon dioxide of $10^{-3.5}$ atm for atmospheric conditions, the unbalanced reaction

$$2H^+ + CO_3^{2-} \rightleftharpoons H_2O \qquad ...3.24$$

with a conditional constant as defined by (30) of logK' = 21.65. This is the form of reaction 3.22 as required by the input to MINEQL.

The values reported for the solubility product of calcium carbonate do not vary greatly. A value for logK_{sp} of 8.49 (102) was thus chosen, as this was determined using three experimental techniques simultaneously and compares favourably with other values given in the literature (103, 104, 85).

3.3.2.4 Redox speciation and the equilibria of dissolved oxygen

The nutrient solutions in this study were initially modelled in equilibrium with atmospheric oxygen, thus using the oxygen redox couple to distribute the other components amongst their redox species according to the relevant half-cell reactions. This was done by incorporating the oxygen redox half reaction

$$^1/_2\ H_2O \rightleftharpoons {}^1/_4\ O_{2(aq)} + H^+ + e^- \qquad ...3.25$$

into the model with a logK value of -21.50. By Henry's law on dissolved gases, using a value of $10^{-2.89}$ ( ) for the solubility of oxygen in aqueous solutions, i.e. $O_2(aq)/O_2(g)$ is equal to $10^{-2.89}$, the following reaction was obtained

$$^1/_2\ H_2O \rightleftharpoons\ ^1/_4\ O_2(g) + H^+ + e^- \qquad \text{...3.26}$$

with a log $K_{(g)}$ of -20.78. Assuming then a constant partial pressure of 0.2095 atm for oxygen in the atmosphere this gave the reaction

$$^1/_2\ H_2O \rightleftharpoons H^+ + e^- \qquad \text{...3.27}$$

with the conditional constant as defined in reference (30) of log K' = -20.63. Combining this with the other redox half reactions, for example with that of iron

$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+} \qquad \text{...3.28}$$

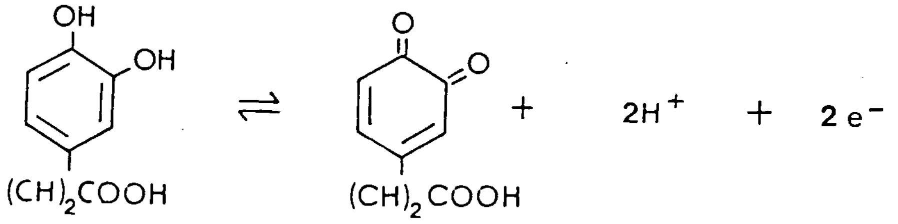
which has a logK value of 13.02, the following reaction was obtained

$$Fe^{3+} \rightleftharpoons Fe^{2+} + H^+ \qquad \text{...3.29}$$

with the conditional constant logK' = -7.61 as used in the input to the program MINEQL.

3.3.2.5 The caffeic acid redox reaction.

A value for logK of 13.68 was used for the half-cell redox reaction of caffeic acid



...3.30

This was calculated from the redox potential reported by Horner and Geyer (25) for the half-cell reaction of 0.792 volt, using equation 3.5.

3.3.3 Temperature and ionic strength adjustments

The temperature chosen for the nutrient solution model was 25°C, as it was thought that this would represent a reasonably good mean value for plant growth. As most constants reported in literature were determined at this temperature, adjustments of the stability constants for temperature were only required in a few instances. This was almost always only for small adjustments of up to 5°C. Hence the assumption of constant $\Delta H^{\ominus}_{m}$ with temperature could generally be justified. Once again these adjustments were mainly carried out only as a check on the equilibrium constants reported at 25°C, when the thermodynamic data were available.

It was decided not to fix a specific value for the ionic strength to be used in the building up of the nutrient solution model, but to calculate this iteratively for each of the solutions modelled. Thus the model was initially constructed with all constants adjusted to an ionic strength of 0.0 mol dm^{-3} by the methods outlined in section 3.2 using LOGK for aqueous complex equilibria (28) and LOGKSP for the solubility products.

The final model as used in this study is given in Appendix A in the form of the input as required by the program MINEQL. This consists of 270 aqueous equilibria and 39 potential solids, which are all written in the form of formation reactions. Futhermore MINEQL does not consider the hydroxy ion as a separate component and therefore all reactions are written in the form of proton input only. The above conventions are best illustrated by the following set of reactions for the input of the solubility product of zinc hydroxide, which is given by the reaction

$$Zn(OH)_2(s) \rightleftharpoons Zn^{2+} + 2OH^- \qquad \Longrightarrow \log K_{sp} = -15.52$$

This can be written in the form of a formation reaction, i.e.

$$Zn^{2+} + 2OH^- \rightleftharpoons Zn(OH)_2(s) \qquad ==> \log K = 15.52$$

and gives by addition of twice the dissociation product of water, the following

$$Zn^{2+} + 2H_2O \rightleftharpoons Zn(OH)_2 + 2H^+$$

with a logK = -12.48 at 0.0 mol dm^{-3} ionic strength and 25°C. This leads to the input to MINEQL, with the particular systematic number for the solid of 81204, and component numbers for Zn^{2+} and H^+ of 12 and 50 respectively :

```
81204      -12.48 12 1 50 -2
```

For each new solution the ionic strength was iteratively computed requiring about 3 cycles before a final value was obtained at which all further modelling was carried out for that particular solution. As it was very tedious to change the roughly 250 constants each time to the more refined ionic strength, use was initially made of the adjustment facility incorporated in MINEQL. This is based on a form of the extended Debye-Hückel equation as proposed by Davis (101). This procedure made quite consistent adjustments with those obtained using LOGK and LOGKSP, which is probably due to the small ionic strength differences from 0.0 mol dm^{-3} to usually less than 0.015 mol dm^{-3}, for which the corrections were made.

At this stage the model was obviously not considered to be complete or final. Preliminary work had to be carried out for purposes of validation and subsequent refinement. Modelling is in any respect an ongoing process as different questions are asked, additional equilibria are required and kinetic factors coming to our attention are considered. Therefore continuous refinement of constants and adjustments to the approaches used in the model are required to be made. Thus at no time can the work really be considered to be complete.

3.4 Validation

The validation of the model was primarily based on correspondence between predicted pH and the reported observed pH for given nutrient solutions. These results are given in Table 3.2 for a number of nutrient solutions modelled, using the final constants after refinement, given in Appendix A.

Although these represent quite different compositions these all gave rather good correspondence for solutions with low pH, as can be seen from Table 3.2. However for pH > 6.5 the computed values were often found to be about 0.8 of a log unit greater than the measured pH's.

At this stage it should be pointed out that the pH calculated by MINEQL is equal to the negative logarithm of the free hydrogen ion concentration, because the program considers concentration equilibria only. This should obviously therefore, not be directly compared with the experimentally determined operational pH, as defined in section 2.2, which is a measure of the hydrogen ion activity. By definition the activity is only equal to the concentration at conditions of infinite dilution. However the ionic strengths as calculated from the speciation results of the nutrient solutions modelled in this study was generally found to be in the range of 0.01 to 0.015 mol dm^{-3}. In this ionic strength range, Kielland suggests a value of 0.93 for the activity coefficient of the hydrogen ion (78). Thus considering the ionic strength, a value for the measured pH of for instance, 5.00 would be equal to 4.97 for the negative log of the hydrogen ion concentration. This difference of 0.03 is less than the generally accepted uncertainty in pH measurements of ±0.05 of a log unit (33).

Table 3.2

solution type	reference used for composition	measured pH as per reference	computed pH using the model
Modified Steinberg	105	6.0	5.98
Modified 20% Johnson	106	6.1	6.16
Modified Hoagland no.1	5	5.2 7.5*	5.14 8.1 -> 7.5#
Johnson	107	4.5 - 5.0	4.9
--	108	4.4 7.4 - 7.6	4.37 8.0 -> 7.5#
Long Ashton	80	5.5 - 6.0	5.48

* obtained by the addition of solid calcium carbonate resulting in solutions saturated with respect to calcium and carbonate ions.

\# shift obtained in modelled pH on consideration of various kinetic factors.

3.4.1 Considerations of the carbonate system.

Several standard buffers were modelled and the speciation results used to validate the model. As the ionic strengths were found to be somewhat larger than those of the nutrient solutions, the measured pH values were adjusted for changes in the activity coefficients and very good correspondence with the computed values was found even at high pH. These buffer solutions are very well defined systems but consist of only a few components in each case. They are therefore not a sufficient test of the model as only a small section of the total equilibria are involved. However, a good correspondence at all pH's modelled was observed. This seemed to indicate that the non agreement between computed and actual pH's of the nutrient solutions at high pH was linked to the carbonate problem. This was due to the way in which the condition of high pH is generally obtained in experimental work with plants, i.e. by the addition of solid calcium carbonate. As the values used for the two equilibria involved are thought to be reasonably accurate, it is therefore assumed that kinetic factors might be implicated. Thus for the purposes of refinement further investigation was carried out along these lines.

Plummer et al have extensively studied the kinetics of calcium carbonate, i.e. calcite dissolution by two different experimental methods. This lead to the proposal of a mechanistic model (109, 110, 111) which was successfully tested using the kinetic results of studies by other workers on the dissolution and precipation of calcium carbonate (112).

They proposed the following equation for the nett rate of dissolution determined by the difference in forward and backward reaction rates,

$$R = k_1 a_{(H^+)} + k_2 a_{(H_2CO_3)} + k_3 a_{(H_2O)} - k_4 a_{(Ca^{2+})} a_{(HCO^-)}$$

where 'a' denotes activity of the bulk fluid

$H_2CO_3^* = H_2CO_3^o + CO_2^o$ as before

k_1, k_2 and k_3 are rate constants dependent on temperature, and

k_4 is the backward rate constant dependent on temperature and carbon dioxide partial pressure in a very complex relationship.

The rate dependence suggests that for the forward rate of dissolution 3 reactions occur simultaneously at the calcite surface

$$CaCO_3 + H^+_{(o)} \rightleftharpoons Ca^{2+}_{(s)} + HCO_3^-{}_{(s)} \qquad ...3.31$$

$$CaCO_3 + H_2CO_3^o{}_{(o)} \rightleftharpoons Ca^{2+}_{(s)} + 2HCO_3^-{}_{(s)} \qquad ...3.32$$

$$CaCO_3 + H_2O_{(s)} \rightleftharpoons Ca^{2+}_{(s)} + HCO_3^-{}_{(s)} + OH^-_{(s)} \qquad ...3.33$$

where the subscripts (s) and (o) indicate species in the surface (i.e. adsorption) layer and species in the boundary layer (i.e. = bulk fluid) respectively.

The backward reactions which really represent precipitation of calcite are a function of the activity product $a_{(Ca^{2+})} a_{(HCO^-)}$ and the mechanisms can be represented by the following

$$| Ca^{2+} - HCO_3^- |^+_{(o)} \rightarrow CaCO_3 + H^+_{(s)} \qquad ...3.34$$

$$| Ca^{2+} - HCO_3^- |^+_{(o)} + HCO_3^-{}_{(s)} \rightarrow CaCO_3 \qquad ...3.35$$

and $$| Ca^{2+} - HCO_3^- |^+_{(o)} + OH^-_{(s)} \rightarrow CaCO_3 + H_2O_{(s)} \qquad ...3.36$$

The notation $|Ca^{2+} - HCO_3^-|^+_{(o)}$ points to the uncertainty in the physical nature of the ion association during the backward reaction. Due to the nett positive charge on association it is assumed, from electrostatic considerations, that these processes occur at negatively charged reaction sites. These sites could be occupied by anions, the obvious ones present at the surface of the solid in this system being CO_3^{2-}, HCO_3^- and OH^-. This really leads to the proposal of the three mechanisms for the back reactions given above.

Returning to the dissolution reactions three separate but overlapping regions can be distinguished. Depending on solution conditions one or the other reaction predominates. Thus at high pH and low carbon dioxide partial pressure i.e. pH > 7 and partial pressure < 0.03 atm respectively, reaction 3.33 which involves attack by water on the calcite surface predominates. Plummer et al have shown that only reaction 3.31 is fast and that reactions 3.32 and 3.33 are slow. This is probably why quite slow rates of calcite dissolution in the order of a few milligrams per cm^2 per year are therefore observed for systems with similar conditions to those in the nutrient solutions. Furthermore it has been found that impurities can severely affect rates of calcite dissolution. Impurities can be classed into two groups i.e. common ions and true inhibitors such as phosphate, copper(II) ions, etc. The non calcite derived common ions would cause the calcite equilibrium speciation within the adsorption layer to differ from that in the pure carbon dioxide-water system resulting in decreased solubility and increased precipitation.

In trying to incorporate these kinetic effects into our model the above points suggest that the calcium carbonate solubility product should be changed. In particular during dissolution the ratio Ω defined by

$$\Omega = \frac{\text{activity product}}{\text{solubility product}}$$

is less than 1 which suggests the requirement of decreasing the solubility product. Thus it was found, by trial and error, that a value for the logK of the formation reaction as required by MINEQL of 9.4 resulted in a good correspondence between observed and computed pH's for the high pH nutrient solutions modelled. This represents a change of 1 log unit in the constant.

Alternatively the constant of the atmospheric carbon dioxide equilibrium could be changed, which can be justified by the following arguments. For conditions of low carbon dioxide partial pressure i.e. < 0.03 atm, severe discrepancies between theoretical and experimental rates are observed and the model of Plummer et al cannot be succesfully used. At these conditions the carbonate system is therefore still not fully understood. The assumption that bulk carbon dioxide partial pressure is equal to that in the adsorption layer, on which their model is based, is probably no longer valid. According to Plummer et al (110), this could be due to slow exchange of aqueous carbon dioxide with the gaseous phase on calcite dissolution especially at pH's greater than 7, at which the $H_2CO_3^*$ activity is somewhat less than that of HCO_3^-. Therefore it becomes extremely difficult to buffer the activities of the carbonate species in such solutions, particularly in those that are not generally stirred and through which no gaseous phase is bubbled.

Furthermore, Plummer et al (112) suggest that discrepancies between calculated and observed results could be due to significant variations in the surface partial pressure of carbon dioxide. They calculated that a variation of one order of magnitude would bring about a pH change of 0.66 of a log unit.

Thus if the modelled pH is to be raised the carbon dioxide partial pressure just needs to be increased. Again by trial and error good correspondence between observed and modelled pH was achieved, if the atmospheric partial pressure was increased from $10^{-3.5}$ to about $10^{-2.5}$ atm. This results in a decrease in the logarithm of the gaseous equilibrium constant, for the reaction as used in MINEQL, of about 1 log unit from 21.65 to 20.70. Thus either the solubility product or the gaseous dissolution constant was changed. If the latter method, i.e. of adjusting the carbon dioxide partial pressure is used, then the decreased value of the constant is only applicable in the modelling of high pH solutions obtained by the addition of solid calcium carbonate. Thus the problem was in both cases alleviated only by the empirical approach of adjusting the relevant equilibrium constants whenever solutions saturated with respect to solid calcium carbonate were modelled. This is obviously not a completely satisfactory technique but serves the purposes of the modelling study relatively well. A more involved experimental or theoretical study of the carbonate problem with particular respect to nutrient solutions is however beyond the scope of the present study and was not the principle aim in any event. It is also doubtful whether the considerable effort involved would have been all that worthwhile.

3.4.2 Considerations in the redox approach.

For the purposes of validation of the redox equilibria, it had been intended to compare computed with measured redox potentials of the solutions modelled. Due to severe problems encountered in the measuring of reduction potentials listed in reference (61), few researchers have used Eh measurements other than in a qualitative sense. For the purposes of this discussion Eh can be defined by the following equation given in reference (83)

$$Eh = \frac{2.303RT}{F} \quad pe$$

Redox values, i.e. Eh values, for the nutrient solutions used, were generally not measured or reported, except for a few studies specifically on the effect of redox (92). However Lindsay (83) quotes values for pH + pE of between 2 and 18 as being representative of the reduction potential of soils. Using the constant of logK' = -7.61 for reaction 3.29 this was however found to give a pH + pE value of 20.63 in all modelling results, which is equal to the negative ,logarithm of K' reaction 3.27 and is quite far removed from the pH + pE range as proposed by Lindsay (83). Additionally, this maintained in all instances a very low free iron(II) concentration in the modelled solutions which is contrary to requirements for normal plant growth, see sections 2.1 and 3.5.2.

To model varying pH + pE conditions all that was required was to change the oxygen partial pressure which has the effect of changing the conditional log K' of reaction 3.29. However to obtain modelled pH + pE values at the lower end of the range, i.e. of about 5, required unrealistically low values for the oxygen partial pressure. Nordstrom et al (61) found that dissolved oxygen was indeed in equilibrium with atmospheric oxygen for the systems of turbulent streams investigated. However, they found that the redox state of these waters studied was determined by the iron redox half cell-reaction, and that the Eh values calculated from the amount of dissolved oxygen were considerably higher than those measured. Therefore they concluded that the dissolved oxygen was not in equilibrium with the ferrous-ferric redox couple.

This suggests that some form of kinetic inhibition exists for the reaction

$$Fe^{3+} + {}^{1}/_{2}\,H_2O \rightleftharpoons Fe^{2+} + {}^{1}/_{4}\,O_2(aq) + H^+ \quad ...3.37$$

and that the H_2O/O_2 couple is far from equilibrium in the system except at elevated temperature. This inhibition and the resulting thermodynamic disequilibrium has also been proposed by other workers (113, 114).

Thus modelling in the presence of equation 3.27 leads to results possibly far removed from homogeneous equilibrium to be of any reasonable use. Therefore subsequent modelling was carried out in the absence of equilibrium reactions 3.25 and in turn 3.29. Thus quite simply the redox half-cell reactions, for example 3.28 were used in this study. This requires the theoretical concept of free electrons in solution as discussed by Lindsay (83). The approach to redox speciation is therefore similar to that used in the studies by Ball et al (91). This then gave for most solutions modelled a pH + pE of about 18 which is the value proposed by Lindsay for well aerated soils (14, 83).

The approach used by Ball et al in their study using the program WATEQ2 (91) does not however generally distribute the elements amongst their redox species. If only the one form of an element is used as input, for instance Fe(III), then only Fe(III) speciation is considered unless a redox value is input as well. If both Fe (III) and Fe(II) forms are input a redox value from the computed activity ratio after speciation is calculated and used to distribute the other elements amongst their redox species.

We thus propose that for plant nutrient solutions which are subject to microbial action and/or plant exudates which can both result in changing redox potentials, these should be used to distribute the redox elements amongst their species with the different oxidation states. This is particularly relevant to the speciation of iron, as solutions are generally made up using only the 3+ form. Similarly, the approach used by Ball et al would then require a redox input value to distribute the total iron amongst the redox species, as otherwise no iron(II) speciation would be computed and the speciation results could be far removed from reality.

Thus computed and measured pH's of the solutions with basic conditions obtained by the addition of solid $CaCO_3$, could now be modelled more succesfully. This is not to say that this is the best or only way by which this can be achieved. This particular problem probably still requires further study in the future. It is felt that the problem of redox speciation esspecially for that of iron in plant nutrient solutions is solved relatively well in this study but also still requires experimental validation.

3.5 Results and Discussion

The modelling investigations were performed following the procedure given in the flow diagram, Figure 3.1. To evaluate the results obtained the null hypothesis principle was used wherever possible (37).

The preliminary investigations in this study were directed towards the following problems

1. the effect of excess chelating agent on micronutrient concentrations in plants.

This arose mainly from the experimental work by Wallace et al (118). These modelled systems, as well as several other well defined buffer solutions were used in the validation of the model on the basis of comparing measured with computed pH.

Then, as the main section of this study, the following investigation was undertaken,

2. the effect of caffeic acid on iron nutrition was investigated,

arising out of the experimental work of Olsen and co-workers (18, 23, Marschner et al (10) and Tipton and Thowson (21).

3.5.1 The effect of excess chelating agent on micronutrient concentration in plants

Wallace and co-workers in various biological experiments with plants grown in nutrient solutions have investigated many different factors affecting plant nutrition (115, 116). One of these is the effect that excess chelating agent has on the micronutrient uptake by plants (117, 118, 119, 120). The term excess is used here because the chelating agents were added to the solutions in the uncomplexed form, i.e. as the respective sodium salts, in excess of the amount added primarily in the form of the iron(III) complex. Closely related to this work on excess ligand concentation is the study on the use of more than one chelating agent (108).

In a study of bush beans grown in nutrient solutions Wallace found that high levels of DTPA decreased plant yields (118). This he proposed to be due to copper deficiency at low pH, based on the very low copper concentration measured in the plants. Furthermore, Wallace proposed that, at high pH, reduced plant yields were caused by copper, manganese, and zinc deficiencies. The argument was again based on low concentrations of the nutrients in the plants. Zinc uptake was found to decrease at both pH's while manganese uptake was only really affected by DTPA at high pH. Similarly it was found that excess EDTA significantly decreases the copper concentrations of the plants studied at low pH, but caused only slightly lower zinc and manganese concentrations. No adverse effects of EDTA were observed at high pH. Wallace found (118) that excess EDDHA had little or no effect on the uptake of manganese, copper, and zinc(II). This has also been reported in other studies by Wallace (120).

As a reason for the observed decreases in metal concentrations of the plants Wallace proposed the hypothesis that this was due to increased complexation, resulting in competion between the roots and the chelating agent for the micronutrients. Various workers have suggested that the respective free aquo ions of the micronutrients, including iron, are the bioavailable forms required by plants for normal growth (121, 92, 122, 123). These findings thus support the above chelate uptake inhibition theory. It is in sharp contrast therefore that decreased iron uptake is not observed for EDDHA, which is a much more powerful iron chelator than DTPA or EDTA. This ligand has in fact been shown to increase yields in some instances, apparently due to increased iron uptake. Iron nutrition therefore shows anomalous results and will not be discussed here. This is dealt with in section 3.5.2 on the effects of exuded compounds, and the anomalies are discussed in Chapter 4, which is the general discussion.

In an attempt to determine the possible reasons for the afore-mentioned findings on the manganese, copper and zinc nutrition, the chemical model constructed in the previous section 3.3 was used to investigate the effects of excess chelating agent on micronutrient speciation at low and high pH. The nutrient solution chosen was that used by Wallace et al in their experiments with plants on the use of more than one chelating agent (108). The composition, of this is given in Table 3.3. This was modelled in the presence of varying concentrations of excess DTPA or EDTA as indicated in the table. The following null hypothesis was formulated to facilitate the interpretation of the speciation results obtained.

Table 3.3

Composition of the nutrient solution used to study the effect of excess chelating agent on micronutrient speciation.

Components	Concentration (mol dm^{-3})	Components	Concentration (mol dm^{-3})
Ca^{2+}	*2.50 E-3	Mg^{2+}	1.00 E-3
K^{+}	5.00 E-3	Na^{+}	#various
Fe^{3+}	2.00 E-5	Fe^{2+}	0.00
Mn^{2+}	1.00 E-5	Cu^{2+}	1.00 E-6
Zn^{2+}	4.60 E-6	Cu^{+}	0.00
Mn^{3+}	0.00	H^{+}	#various
e^{-}	0.00	$CO_3{}^{2-}$	*0.00
$SO_4{}^{2-}$	3.00 E-3	Cl^{-}	1.00 E-3
NH_3	1.00 E-3	$PO_4{}^{3-}$	1.00 E-3
$BOH_4{}^{-}$	6.00 E-6	$MoO_4{}^{-}$	3.00 E-8
$NO_3{}^{-}$	6.00 E-3	EDDHA	2.00 E-5
$^{a}EDTA^{4-}$	range from 0 to 5.00 E-4		
$^{a}DTPA^{5-}$	range from 0 to 1.00 E-3		

[a] either $EDTA^{4-}$ or $DTPA^{5-}$ was added to a given solution

\# varied according to the amount of the respective sodium salt of the ligands DTPA and EDTA added

* for the high pH condition 3 10^{-2} mol dm^{-3} was added to the concentrations of Ca^{2+} and $CO_3{}^{2-}$

"The effects observed in the biological experiments are not due to changes in the speciation of the relevant metal ions." ...3.1

Stated in this way the null hypothesis questions the assumption that speciation changes manifest themselves in the plant. This modelling study is based almost entirely on this assumption which might not always be applicable and therefore worth investigating. The speciation results in this section are presented in the form of histograms, with the logarithms of the concentration of relevant species on the Y axis and various excess ligand concentrations represented on the X axis.

The modelling was carried out at two different pH's, computed to be about 4.3 and 7.5. These compare very well with the experimentally determined pH values of 4.3 to 4.5 and 7.4 to 7.6, as reported by Wallace (108, 118) and lend some confidence to the validity of the model. The higher pH value was obtained here by the addition of solid calcium carbonate as in the plant experiments.

3.5.1.1 Excess DTPA

First the results with DTPA as the excess ligand are considered. Figures 3.2 and 3.5 represent the free copper(II) aquo ion and zinc(II) aquo ion concentrations respectively in the presence of various DTPA excesses. From these it can be seen that this ligand has a large effect on the free aquo ion concentration, especially on that of copper at high pH. From Figure 3.8 it is observed that excess DTPA affects the manganese(II) concentration only slightly at low pH. However at high pH, Figure 3.9, the free ion concentration is also markedly affected.

Figure 3.2

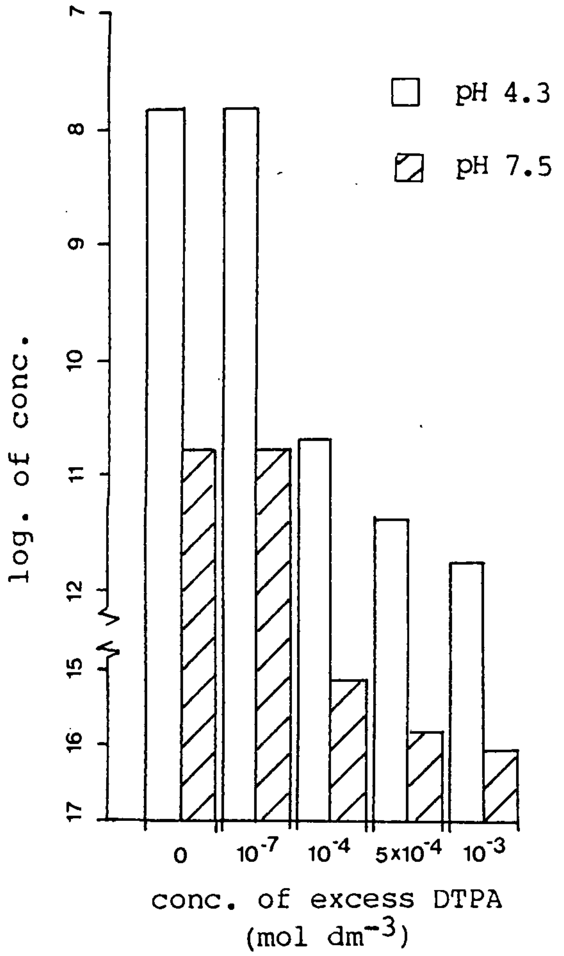


Figure 3.2 Logarithm of the free copper(II) conc. at pH 4.3 and pH 7.5, in the presence of various DTPA excesses.

Figures 3.3 and 3.4 Logarithms of the total concs. of the Cu(II)-EDDHA and Cu(II)-DTPA complexes in the presence of various DTPA excesses, at pH 4.3 and ph 7.5 respectively.

☐ all copper(II)-EDDHA complexes

▤ all copper(II)-DTPA compexes

Figure 3.3, pH 4.3

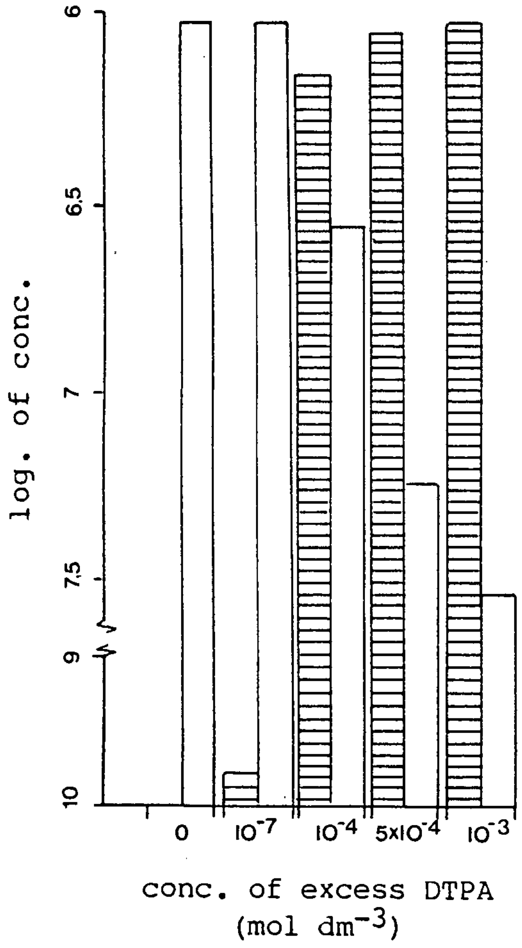


Figure 3.4, pH 7.5

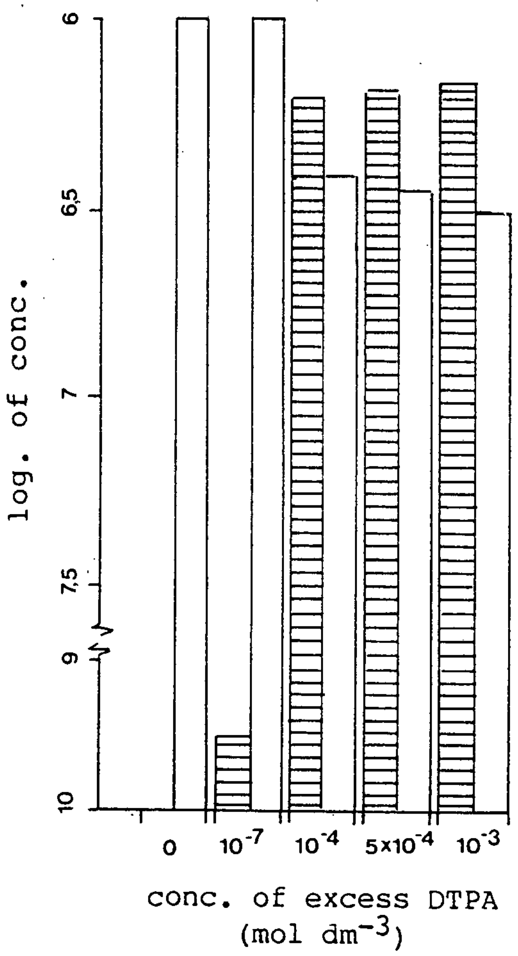


Figure 3.5

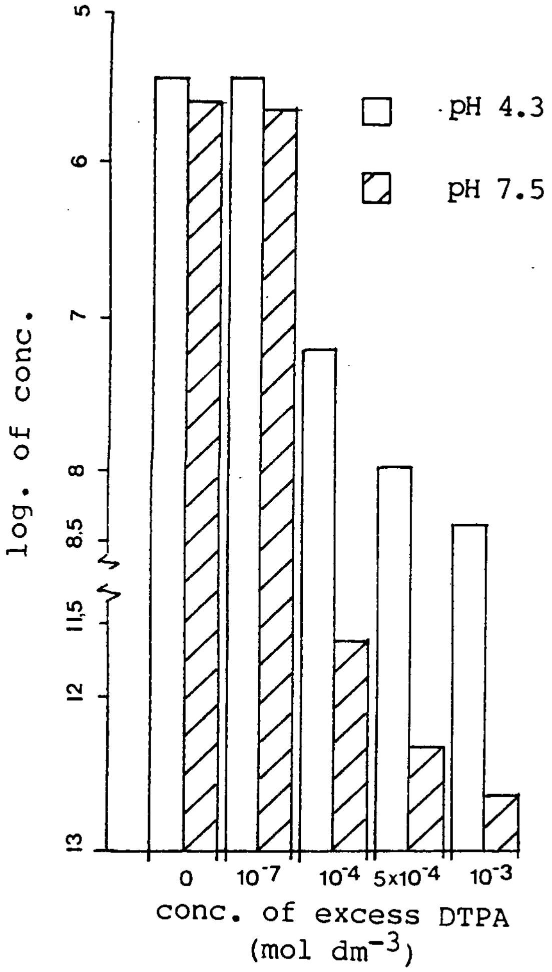


Figure 3.5 Logarithm of the free zinc(II) conc. at pH 4.3 and Ph 7.5 in the presence of various DTPA excesses.

Figures 3.6 and 3.7 Logarithms of the total concs. of the Zn(II)-EDDHA and Zn(II)-DTPA complexes in the presence of various DTPA excesses, at pH 4.3 and ph 7.5 respectively.

☐ all zinc(II)-EDDHA complexes

▤ all zinc(II)-DTPA complexes

Figure 3.6 pH 4.3

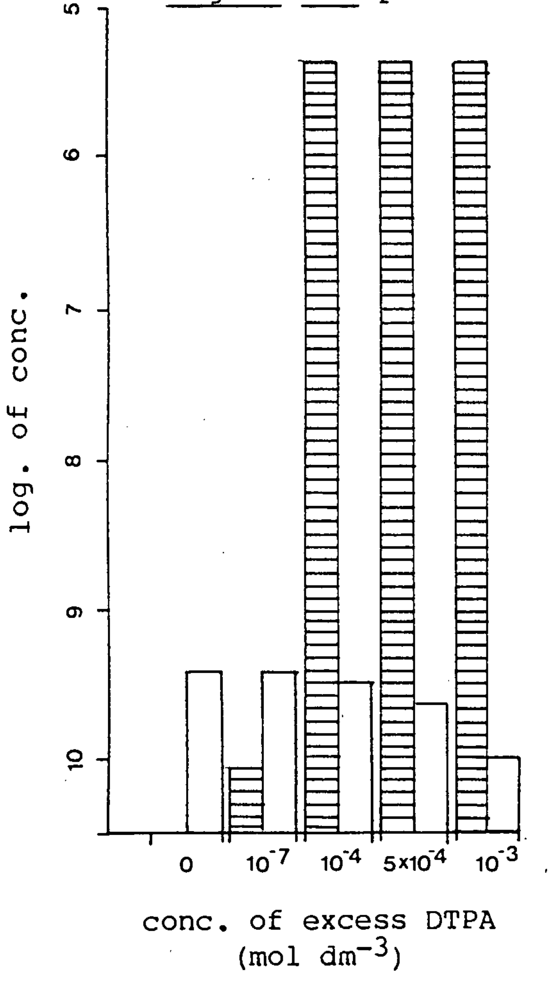


Figure 3.7 ph 7.5

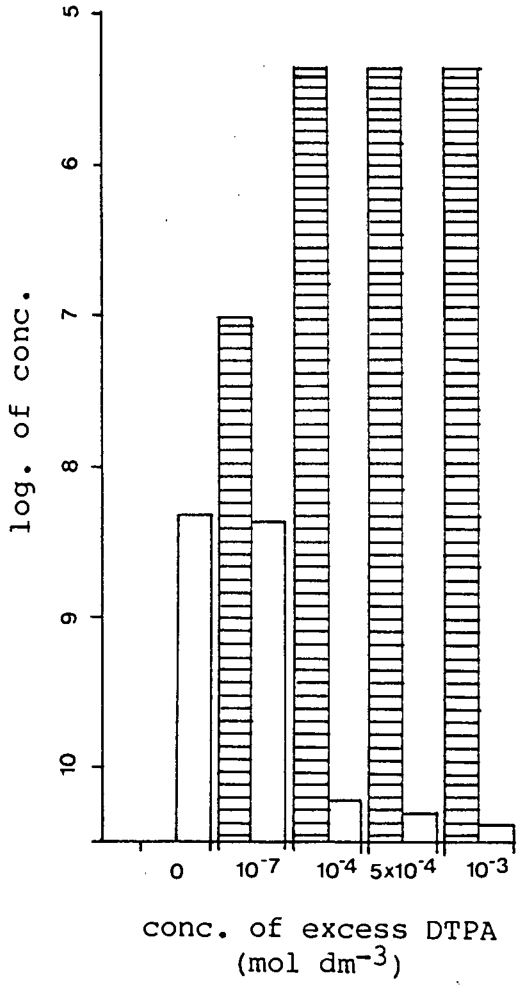


Figure 3.8 Logarithms of the concs. of manganese(II) species in the presence of various DTPA excesses at ph 4.3.

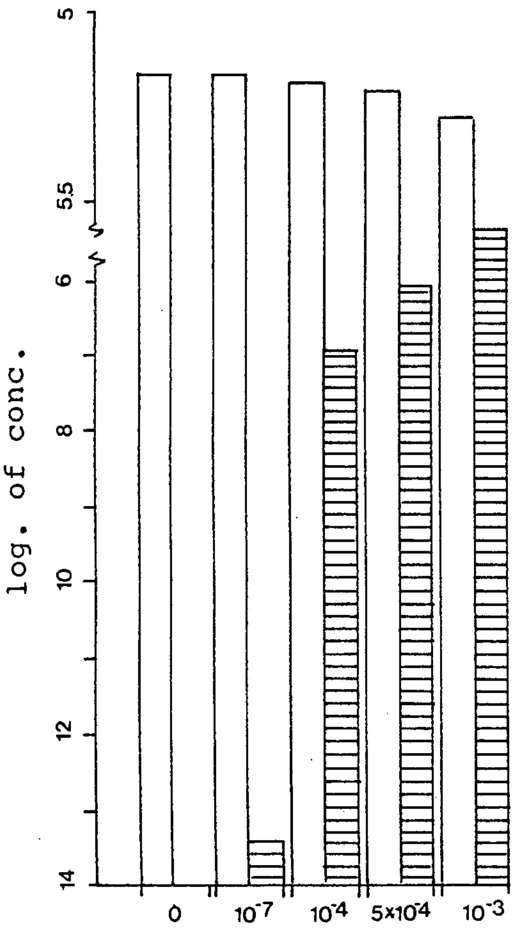
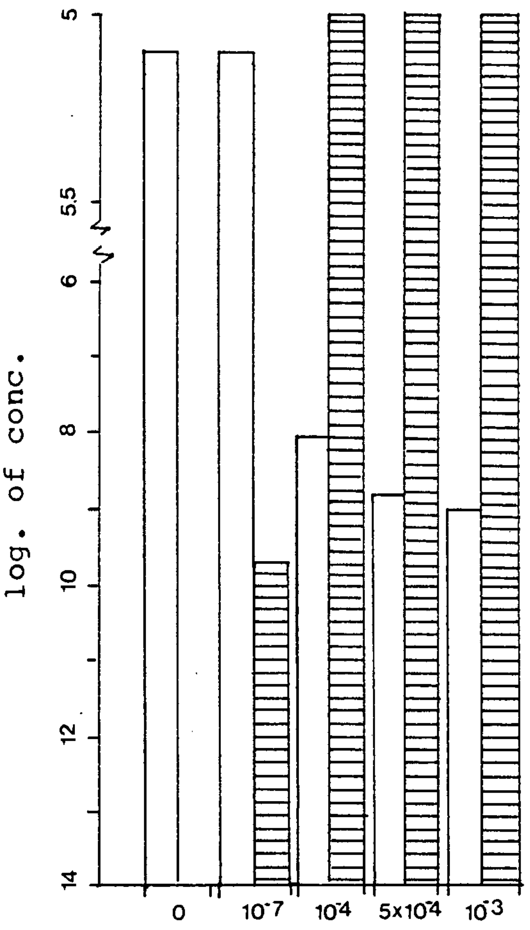


Figure 3.9 Logarithms of the concs. of manganeses(II) species in the presence of various DTPA excesses at pH 7.5.



☐ free manganese(II) aquo ion

▤ all manganese(II)-DTPA complexes

Thus these results can be used to reject the null hypothesis 3.1, as very good correlation between the concentration of the relevant species in solution and uptake by plants is indicated. Furthermore, DTPA seems to be the direct cause of these observations. It would appear therefore that in the case of DTPA speciation changes are involved as factors affecting nutrient uptake by plants. Futhermore, these results are consistent with the view (121, 122) that the free metal aquo ions are the bioavailable forms of these micronutrients required by plants for normal growth.

Considering the copper(II) speciation results in greater detail, it is evident that large decreases in free copper(II) concentration are accompanied by the addition of DTPA. It is also seen from Figure 3.2, that the control, i.e. the solution without DTPA, has roughly the same free copper(II) concentration at high pH as the 10^{-4} mol dm^{-3} DTPA excess at low pH which is about $10^{-10.8}$ mol dm^{-3}. This latter condition is therefore not expected to result in copper deficiency although deficiencies are observed for the 5 x 10^{-4} and 10^{-3} mol dm^{-3} DTPA excesses at low pH conditions. Thus it could be proposed that a lower limit for the free copper concentration required by these plants lies between $10^{-10.8}$ and $10^{-11.4}$ mol dm^{-3}, the latter value representing the maximum concentration computed for deficient plants.

Reference to Figure 3.5 shows that the zinc(II) aquo ion concentrations at low pH are greater than $10^{-8.5}$ mol dm^{-3} throughout. This is somewhat higher than the minimum value of $10^{-10.6}$ as suggested by Lindsay (121). Therefore no zinc deficiency is expected here. However, at high pH with DTPA excesses greater than 10^{-4} mol dm^{-3}, this free concentration falls below the Lindsay limit, suggesting that deficiency may be xindicated due to insufficient zinc. As can be seen from the histogram for manganese, Figure 3.9, the free concentrations remain large throughout at low pH, decreasing only very slightly. This correlates well with the rather constant uptake of manganese by the plant observed by Wallace. At high pH, Figure 3.9, a more marked decrease is observed. However we feel that the concentrations maintained are not small enough to constitute conditions of manganese deficiency.

To determine the way in which the ligand DTPA affects the free micronutrient concentrations in solution and thereby the uptake of the nutrients by plants the following more specific null hypothesis was formulated. This could be done on the basis of prior knowledge indicated by the chelation inhibition theory:

> "Changes in the free metal ion concentration are not brought about by increased excess DTPA through increased chelation." ...3.2

Thus the degree of complexation was then investigated using the same modelling experiments as for rejection of hypothesis 3.1. These results are presented, again in the form of histograms, with the logarithm of the total concentrations of the metal-DTPA and metal-EDDHA complexes on the Y axis in the the presence of various excess concentrations of DTPA along the X axis, modelled at low and high pH.

Considering Figures 3.3 and 3.4 at low and high pH respectively, it can be seen that increasing total concentrations of the copper(II)-DTPA complexes correlate well with the decreasing free copper(II) aquo ion concentration, observed in Figure 3.2. EDDHA complexes much more copper at high pH than at low pH competing well with DTPA. This probably causes the additional decrease in free copper(II) concentration observed in Figure 3.2 with increasing pH, as it is indicated by comparison of Figures 3.3 and 3.4 that DTPA does not in fact complex more copper at high pH.

Considering Figures 3.6 and 3.7 for zinc at low and high pH respectively the trends seem not as clear as observed with copper. (However, the same reasons for decreased free concentrations still apply.) What makes this less evident is the fact that in the presence of concentrations of DTPA in excess of total zinc, it is indicated that all the zinc is complexed at low as well as high pH. Thus the decreasing free zinc concentration observed, in Figure 3.5, does not correlate too well with increasing DTPA excess. This can be explained on the basis of increased strength of complexation. This argument has been suggested by other workers (124) and is discussed in Chapter 4, in another context. Only for the 10^{-7} mol dm^{-3} excess is the zinc partially complexed at both pH's. From the control i.e. no DTPA, and the 10^{-7} mol dm^{-3} excess, it is seen from Figures 3.6 and 3.7 that EDDHA complexes more zinc at high pH which could be a partial reason for the reduced free concentration observed in Figure 3.5 with increasing pH at respective DTPA excesses. However, EDDHA does not seem to be able to compete so well with DTPA at the greater ligand excesses, which results in decreased total zinc-EDDHA complex concentrations.

The absence of any manganese-EDDHA speciation results in Figures 3.8 and 3.9 is due to a paucity in literature data for complexes of this system. As time did not permit , this situation could not be rectified in the present study. However it has been stated that manganese(II) is rapidly oxidized by EDDHA and would therefore only exist for a short duration. This then is an obvious area in which the model needs to be further refined in a future study.

Similar trends as in the copper speciation are however observed for manganese, i.e. increasing complexation results in decreasing free aquo ion concentration at both pH's. However, the effect at low pH is not as pronounced as at high pH or as for the other metals studied. In fact this is the only situation in which the total concentration of the complexes is always less than the free ion concentration. Thus probably, only the very small effect on the free ion concentration is observed in Figure 3.8. The histogram at high pH Figure 3.9, for the manganese DTPA complexation is very similar to that of zinc, i.e. for DTPA excesses greater than 10^{-7} mol dm^{-3}, total complexation seems to be indicated.

Thus on the whole the increased complexation by the ligand DTPA and in some cases EDDHA seems to be the reason for the observed decreases in the free concentrations of the metal ions copper(II), zinc(II) and manganese(II). Therefore, on the basis of these results the above null hypothesis 3.2 can be rejected.

3.5.1.2 Excess EDTA

Next the results with EDTA as excess ligand are considered. In this case the solution in Table 3.3 was modelled in the presence of only the two excesses of 10^{-4} and $5x10^{-4}$ mol dm^{-3} as the plant experiments were also carried out for these conditions only.

The results are again presented in the form of histograms indicating the logarithms of the concentrations of the relevant species in solution, in the presence of various EDTA excesses. Figures 3.10 and 3.11 represent the concentrations of free copper(II) aquo ion and total concentration of the copper(II)-EDTA and copper(II)-EDDHA complexes at pH 4.3 and 7.5 respectively.

From these figures a marked effect on the copper(II) aquo

Figure 3.10 Logarithms of the concs. of the copper(II) aquo ion and the total copper(II)-EDDHA and -EDTA complexes in the presence of excess EDTA at pH 4.3.

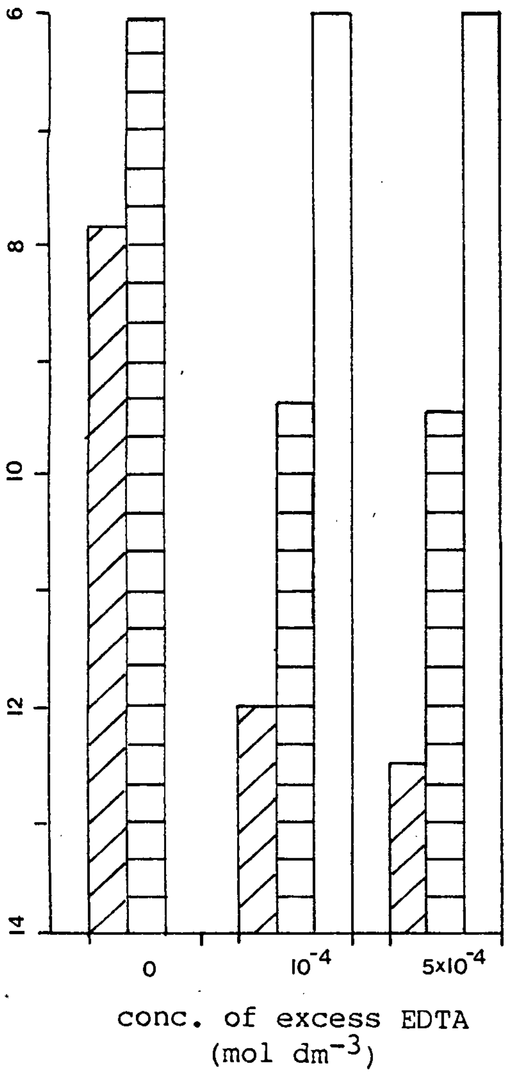
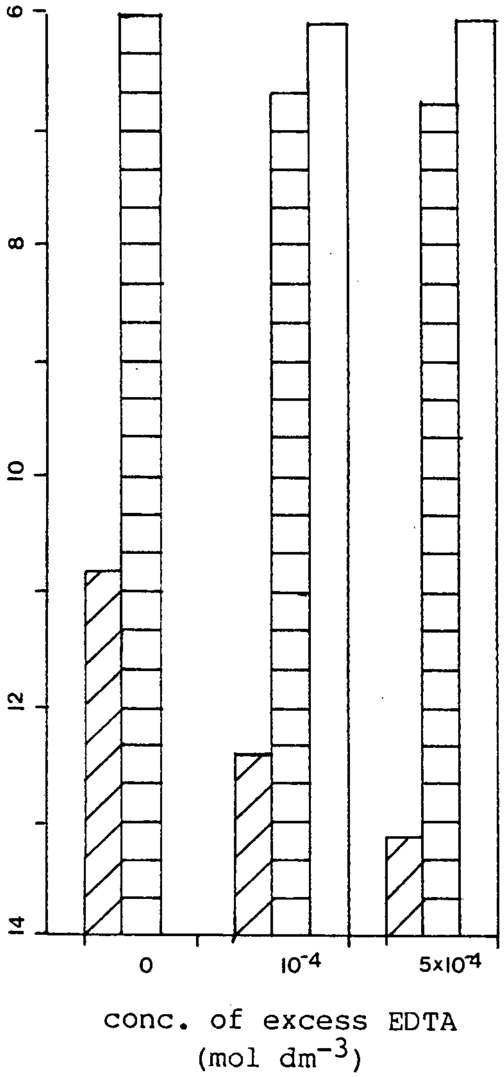


Figure 3.11 Logarithms of the concs. of the copper(II) aquo ion and the total copper(II)-EDDHA and -EDTA complexes in the presence of excess EDTA at pH 7.5.



▨ free copper(II) aquo ion

▤ all copper(II)-EDDHA complexes

□ all copper(II)-EDTA complexes

ion concentrations can be observed at low and high pH. The low pH case Figure 3.10 correlates well with the experimental results reported by Wallace (118) and mentioned at the beginning of section 3.5.1. Decreased free copper(II) concentrations could therefore again be the reason for the lower copper uptake by plants observed with increasing excess ligand in refernce (118). However, for the condition of high pH a slight anomaly is seen. Although EDTA maintains slightly higher free copper concentrations than DTPA, as can be seen on comparing Figure 3.2 with Figures 3.10 and 3.11, these are for all the excess EDTA conditions modelled, still less than the minimum value required for normal plant growth of between $10^{-10.8}$ and $10^{-11.6}$ mol dm^{-3} as proposed from the modelling results in the previous section. Furthermore, the free copper concentration maintained by EDTA, Figure 3.11, is even lower than in the acidic conditions. However, contrary to expectations, the plants were not observed to show signs of copper deficiency. Reasons for this anomaly are discussed in Chapter 4.

As can be seen from Figures 3.12 and 3.13, which represent the concentrations of relevant zinc(II) species in the presence of various EDTA excesses, the free zinc concentration decreases with increasing excess ligand. This correlates well with the decreased zinc uptake observed for plants by Wallace (118). The free zinc concentration maintaned by EDTA does not fall below the minimum value of about $10^{-10.6}$ mol dm^{-3} as proposed by Lindsay in any of the conditions modelled except very slightly for the 5×10^{-4}mol dm^{-3} excess at elevated pH. This is suggested to be the reason why no zinc deficiency was observed in the actual experiments, as was the case with DTPA. If the condition of 10^{-3} mol dm^{-3} excess EDTA had been used to grow plants this might quite possibly have resulted in zinc deficiency especially at high pH, based on the trend observed in Figure 3.13.

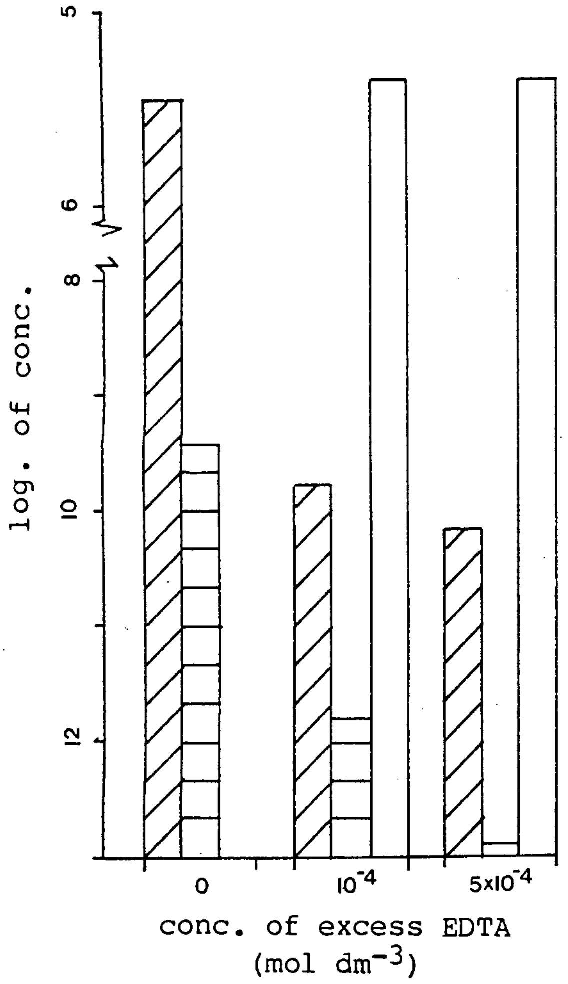


Figure 3.12 Log. of zinc(II) species concs. at pH 4.3 in the presence of various EDTA excesses.

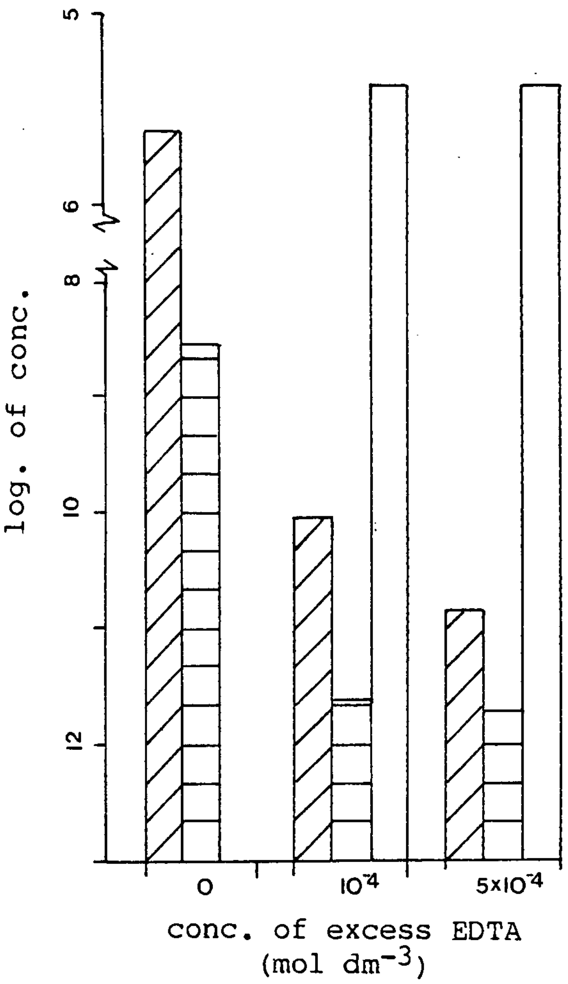


Figure 3.13 Log. of zinc(II) species concs. at pH 7.5 in the presence of various EDTA excesses.

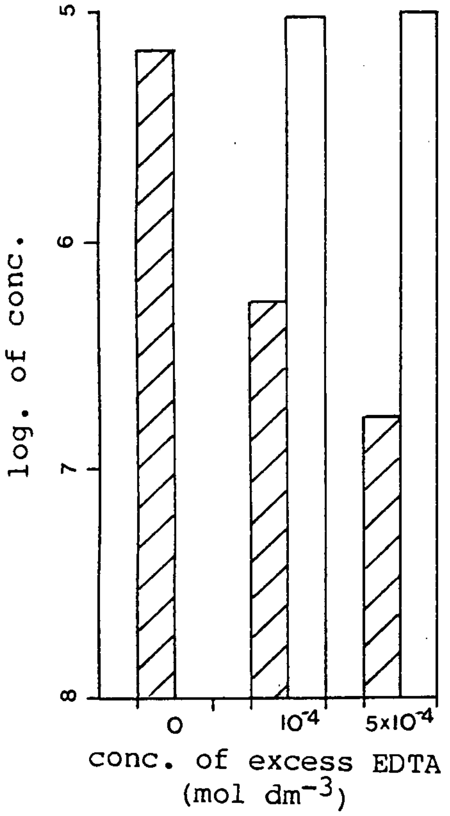


Figure 3.14 Log. of manganese(II) species concs. at pH 4.3 in the presence of various EDTA excesses.

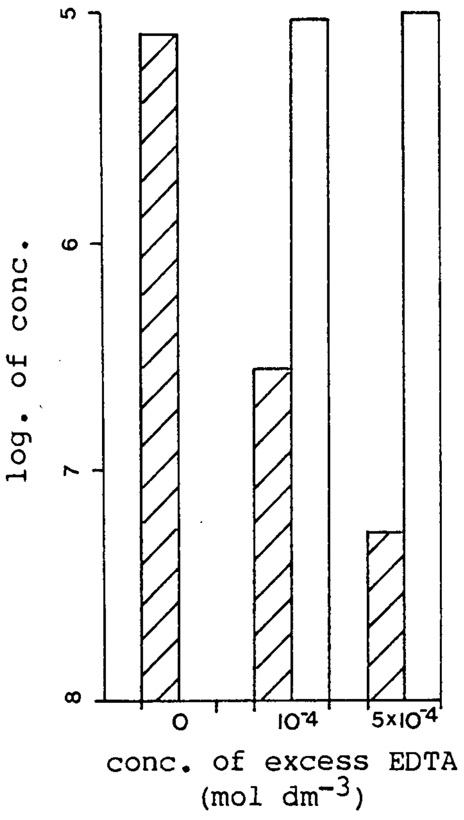


Figure 3.15 Log. of manganese(II) species concs. at pH 7.5 in the presence of various EDTA excesses.

▤ all zinc(II)-EDDHA complexes

☐ all DTPA complexes of zinc(II) or manganese(II)

▨ free aquo ions

The results given in reference (118) suggest slightly decreased manganese uptake with increased excess EDTA, based on measured values of concentration in the plants. This again correlates well with the decrease of free metal aquo ion concentrations as observed in Figures 3.14 and 3.15 representing the speciation results of manganese of relevant species concentrations modelled in the presence of the EDTA excesses. Thus, on the basis of the above results, with only one exception, can null hypothesis 3.1 again be rejected.

In all three instances i.e. with copper(II), zinc(II) and manganese(II) the reduced free metal aquo ion concentrations seem to be a direct result of increased complexation by the excess ligand, in this case EDTA. This is based on similar arguments as used in section 3.5.1.1 for DTPA induced reduction in free aquo ion concentration. Thus the null hypothesis similar to 3.2 but specific to EDTA in this case, which states that changes in the free metal ion concentrations are not brought about by increased EDTA complexation can be rejected.

Therefore, on the whole, the results of this speciation study on the effects of excess DTPA and EDTA on the speciation of manganese, zinc and copper are consistent with the claims made by Wallace (118) and other workers (123), namely that inhibition of micronutrient uptake can result from increased chelate complexation.

Reasons why this does not always seem to be the case, for example as was observed with copper and EDTA at high pH and some other anomalies especially in the nutrition of iron with EDDHA, are discussed in Chapter 4.

As a last point of interest, deficiency conditions were nearly always observed for the situation in which the modelled concentrations of the metal ligand complexes, i.e. with EDDHA and one of the other excess ligands, were both greater than the respective free metal ion concentration.

3.5.2 Caffeic acid modelling

The nutrient solution model, together with the additional equilibrium constants, as determined in an earlier part of this study and reported in the previous chapter, was used to investigate the effect of exuded compounds, in particular the effect of caffeic acid on the micronutrient speciation. The investigation here was mainly conducted from the point of view of iron nutrition by plants, pertinent to the findings of Brown et al, Marschner et al and various other workers already discussed.

The components and their respective concentrations, as initially used in the modelling study are given in Table 3.4, solution B. These represent a 'typical' nutrient solution composition and are based roughly on that used by Tipton and Thowson (21) with the micronutrient concentrations taken from Brown and Olsen (105). However to investigate the effects of the various changing factors on the speciation more clearly, a smaller set of components with slightly different micronutrient concentrations as indicated in Table 3.4, i.e. solution B', was used for a major proportion of the work. All references from here on, if not otherwise indicated, refer to this smaller solution B'.

Table 3.4

Composition of the nutrient solution B and B' used in this study on the effect of caffeic acid together with the total analytical concentrations of the various components given in mol dm^{-3} in the exponential form.

Component	Concentration (mol dm^{-3}) B	B'
Ca^{2+}	1.89 E-3	*--
Mg^{2+}	4.54 E-4	--
K^+	2.06 E-3	#various
Na^+	3.10 E-5	--
Fe^{3+}	3.00 E-5	5.0 E-5
Fe^{2+}	0.00	0.0
Mn^{2+}	6.00 E-6	**1.0 E-5
Cu^{2+}	5.00 E-7	**1.0 E-5
Zn^{2+}	1.50 E-6	**1.0 E-5
Cu^+	0.00	0.0
Mn^{3+}	0.00	0.0
H^+	1.43 E-3	range
e^-	0.00	0.0
$CO_3{}^{2-}$	0.00	0.0
$SO_4{}^{2-}$	4.50 E-4	--
Cl^-	1.80 E-3	--
NH_3	1.20 E-3	--
$PO_4{}^{3-}$	1.30 E-4	--
$EDTA^{4-}$	3.00 E-5	**5.00 E-5
$BOH_4{}^-$	3.00 E-6	--
$MoO_4{}^{2-}$	5.00 E-7	--
$NO_3{}^-$	4.98 E-3	--
$HCaff^{2-}$	5.00 E-5	**1.00 E-4

* Components excluded from solution B'.

\# Varied in accordance with the pH range studied due to KOH addition.

** When included, see text.

3.5.2.1 The effect of caffeic acid

As a means of concisely stating the first problem posed and also as a basis to evaluate the speciation results the following null hypothesis was formulated:

> " Caffeic acid does not affect the iron
> speciation in solution. " ...3.3

The nutrient solution B' was thus modelled with and without caffeic acid in the presence of essentially EDTA, manganese, copper, zinc and iron. The speciation results obtained were plotted in the form of the logarithm of the concentrations versus pH for selected iron species only and are given in Figures 3.16 and 3.17 respectively. Comparing these it can be clearly seen that the presence of caffeic acid significantly influences the speciation, increasing the free iron(II), total iron(II) and iron(II)EDTA concentrations by several orders of magnitude. On the basis of these results the above null hypothesis 3.3 can therefore be rejected, and further investigations carried out.

In order to determine the way in which caffeic acid affects the iron(II) speciation the following extended form of the previous null hypothesis was formulated:

> " Caffeic acid does not affect the iron speciation in solution
> i) through complexation and/or
> ii) through increased reduction of iron(III)
> to iron(II). " ...3.4

Modelling of the same solution B', as before with the ligand present but in the absence of the caffeic acid reduction half-reaction was then carried out, resulting in the speciation curves

Figure 3.16 Speciation diagram of solution B' showing only some of the iron species as discussed in the text.

Figure 3.17 Speciation diagram of solution B', modelled in the absence of caffeic acid.

See Appendix B for the abbreviations used in these figures.

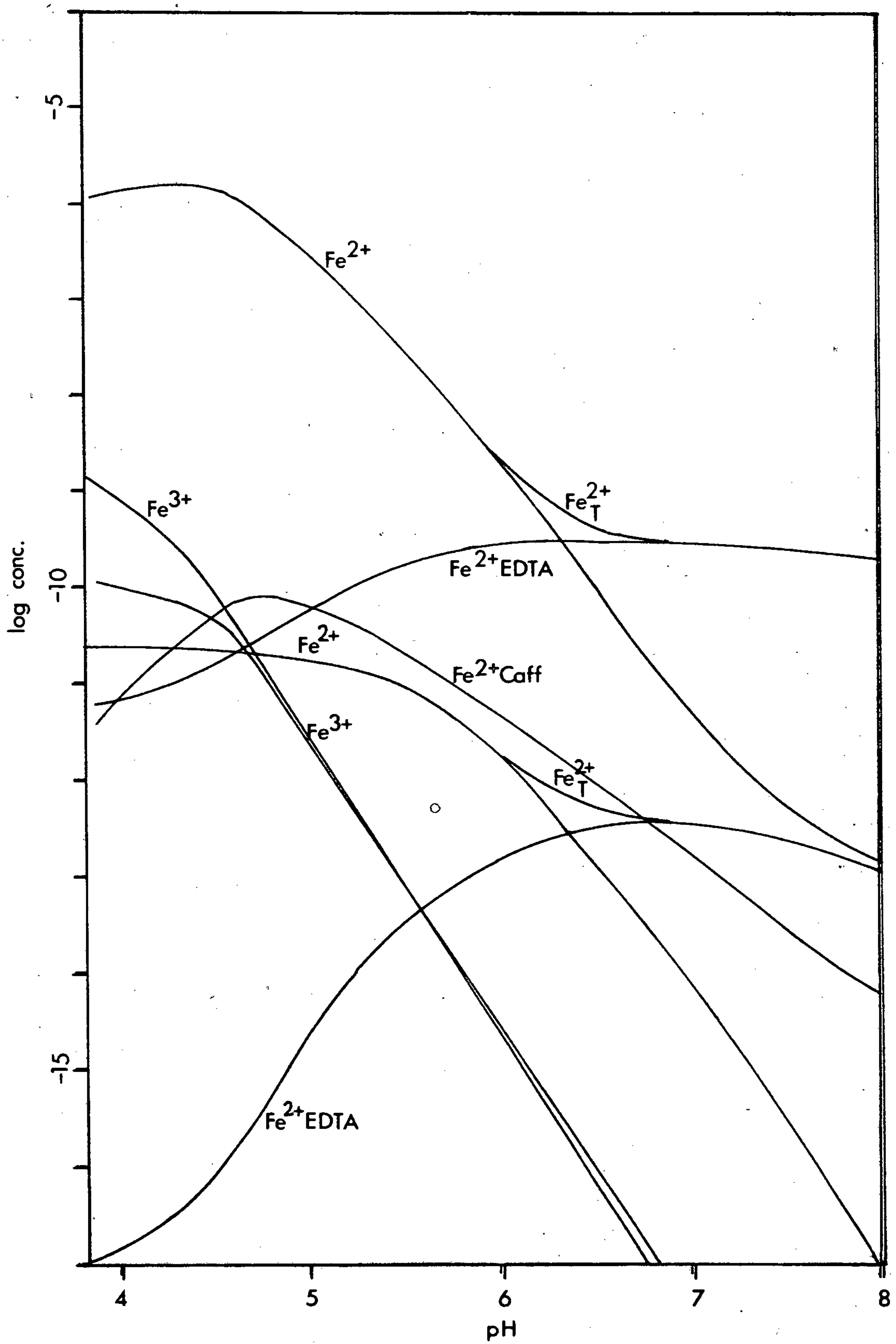
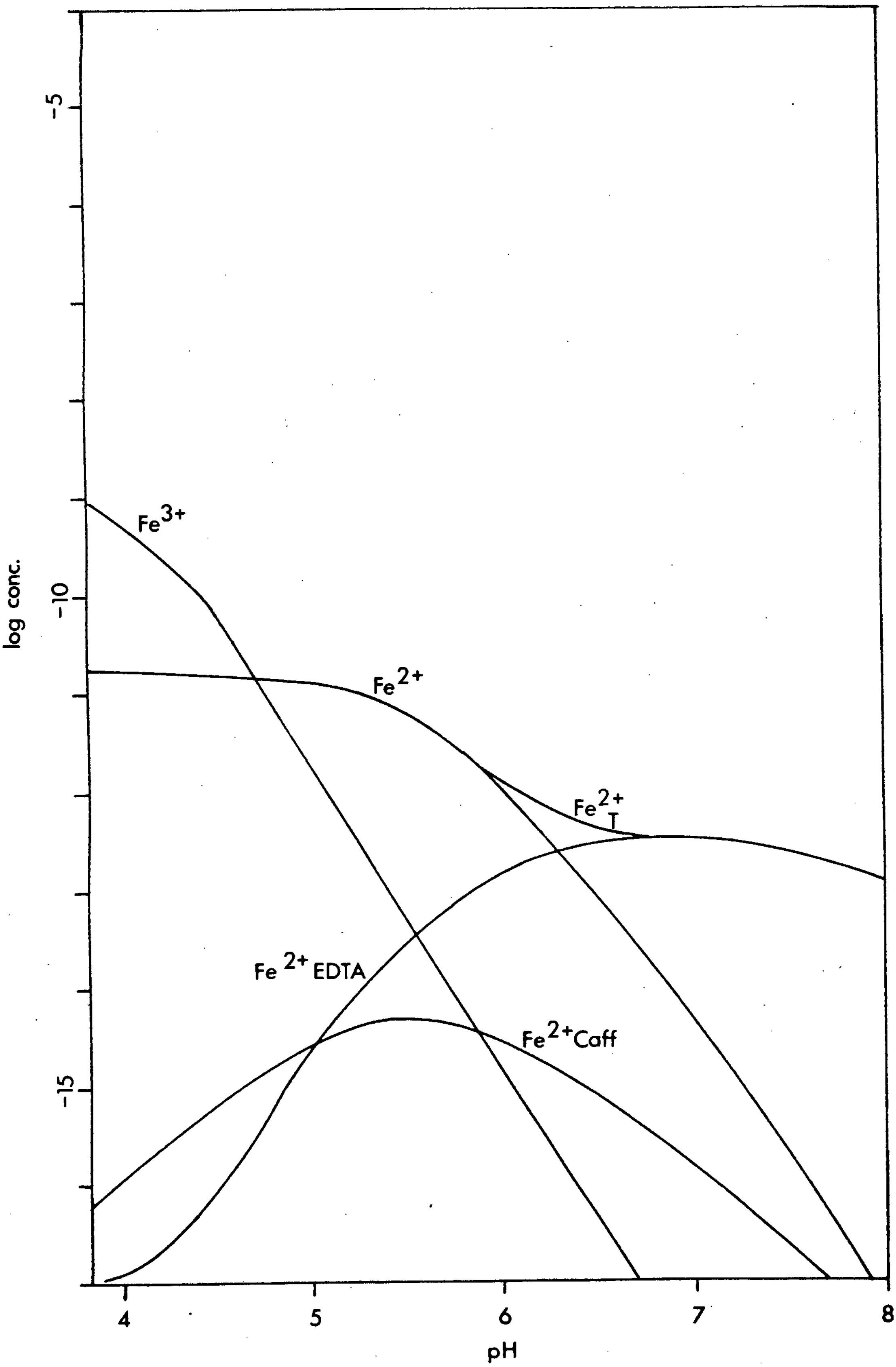


Figure 3.18 Speciation diagram of solution B' modelled in the absence of the caffeic acid reduction half reaction.

See Appendix B for the abbreviations used in this figure.



given in Figure 3.18. Apart from the iron(II) caffeate species, this is evidently very similar to Figure 3.17 and therefore hypothesis 3.4(i) cannot be rejected. This in turn requires rejection of 3.4(ii) since null hypothesis 3.3 had been rejected previously. Thus the results of this study are consistant with the claim made by Olsen et al (9) that excuded substances increase the iron(II) concentration by increased reduction in the rhizosphere, i.e. in the external fluid bathing the roots.

Due to the rejection of the above hypothesis 3.3, this then led onto the investigation of the second problem posed in this study, which is that of the inhibitory effect of manganese(II), copper(II) and zinc(II) on the reduction by caffeic acid.

3.5.2.2 The effect of manganese, copper and zinc.

From observations made in experiments with plants, Olsen et al report inhibition of the caffeic acid reduction of iron by the micronutrients manganese, copper and zinc (18). With the prior knowledge obtained from the study so far the following more specific null hypothesis could therefore be formulated:

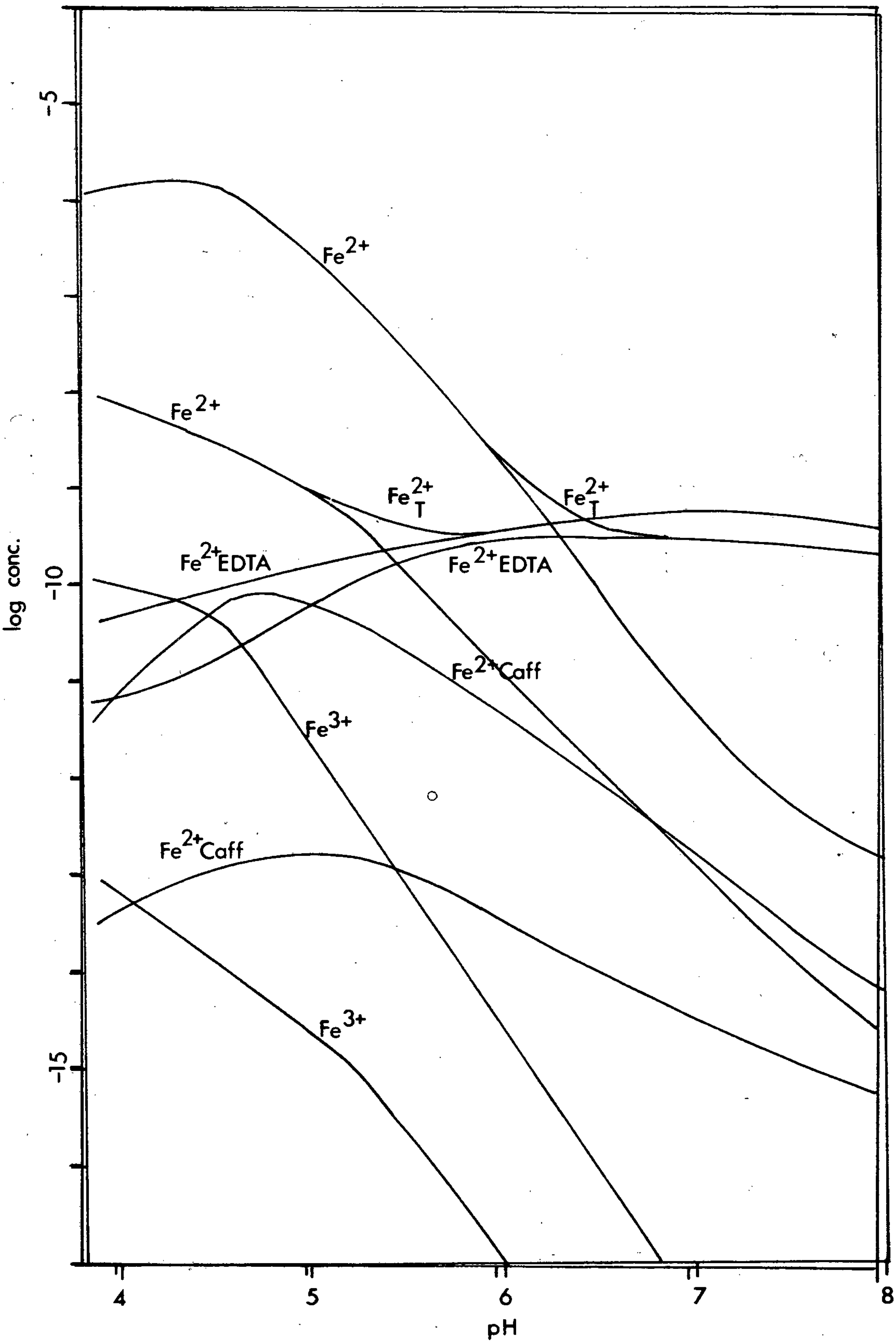
> " Manganese(II), copper(II) and zinc(II) have no inhibitory effect on the increased iron(II) total concentration brought about by caffeic acid. " ...3.5

The solution B' was therefore modelled with the concentrations as indicated in Table 3.4 but in the absence of the proposed inhibitors manganese(II), zinc(II) and copper(II). This resulted in the speciation curves plotted in Figure 3.19. Fig 3.19 is overlayed on Figure 3.16 to facilitate comparison with the speciation of the complete solution B'. The differences observed between these two sets of curves indicates the effect of adding manganese, copper and zinc and at first sight appears to

Figure 3.16 Speciation diagram of solution B' showing only some of the iron species as discussed in the text.

Figure 3.19 Speciation diagram of solution B', modelled in the absence of the metal ions, manganese, copper and zinc.

See Appendix B for the abbreviations used in these figures.



justify rejection of null hypothesis 3.5. However the differences do not actually correspond to inhibition because in the presence of manganese(II), copper(II) and zinc(II) there is an increase in the iron(II) aquo ion concentration. On the other hand, the total iron(II) concentration decreases in the pH range 6 to 8. These effects are attributed to a competitive role of the EDTA, which is discussed in section 3.5.2.3. The lower total iron (II) concentration at pH 6 to 8 shown in Figure 3.19 by comparison with that in Figure 3.16 might possibly be associated with the inhibitory role of the cationic 'inhibitors' reported by Olsen et al (18). In an endeavour to highlight any possible inhibitory effect of the manganese(II), copper(II) or zinc(II), through increased complexation of caffeic acid, a further modelling run was carried out with the caffeic acid concentration reduced to 10 micromol dm^{-3}, but no direct effect on the speciation could be found.

As the speciation results do not on the whole seem to support the findings of Olsen et al on the inhibitory effect of micronutrients on caffeic acid reduction of iron(III), it is suggested that the inhibition observed could possibly be due to interferences in the functioning of the cell membrane or other such related region of effect beyond the scope of this study. Thus the possibility of an enzymatic reduction at specific sites on the root wall occuring as well cannot be ruled out. More research is therefore required in this direction.

3.5.2.3 The effect of EDTA

To investigate the reasons proposed for the effect of the micronutrients on the speciation in section 3.5.2.2, the following null hypothesis was formulated,

" The effects brought about by manganese(II),

copper(II) and zinc(II) on the speciation are
not due to EDTA. " ...3.6

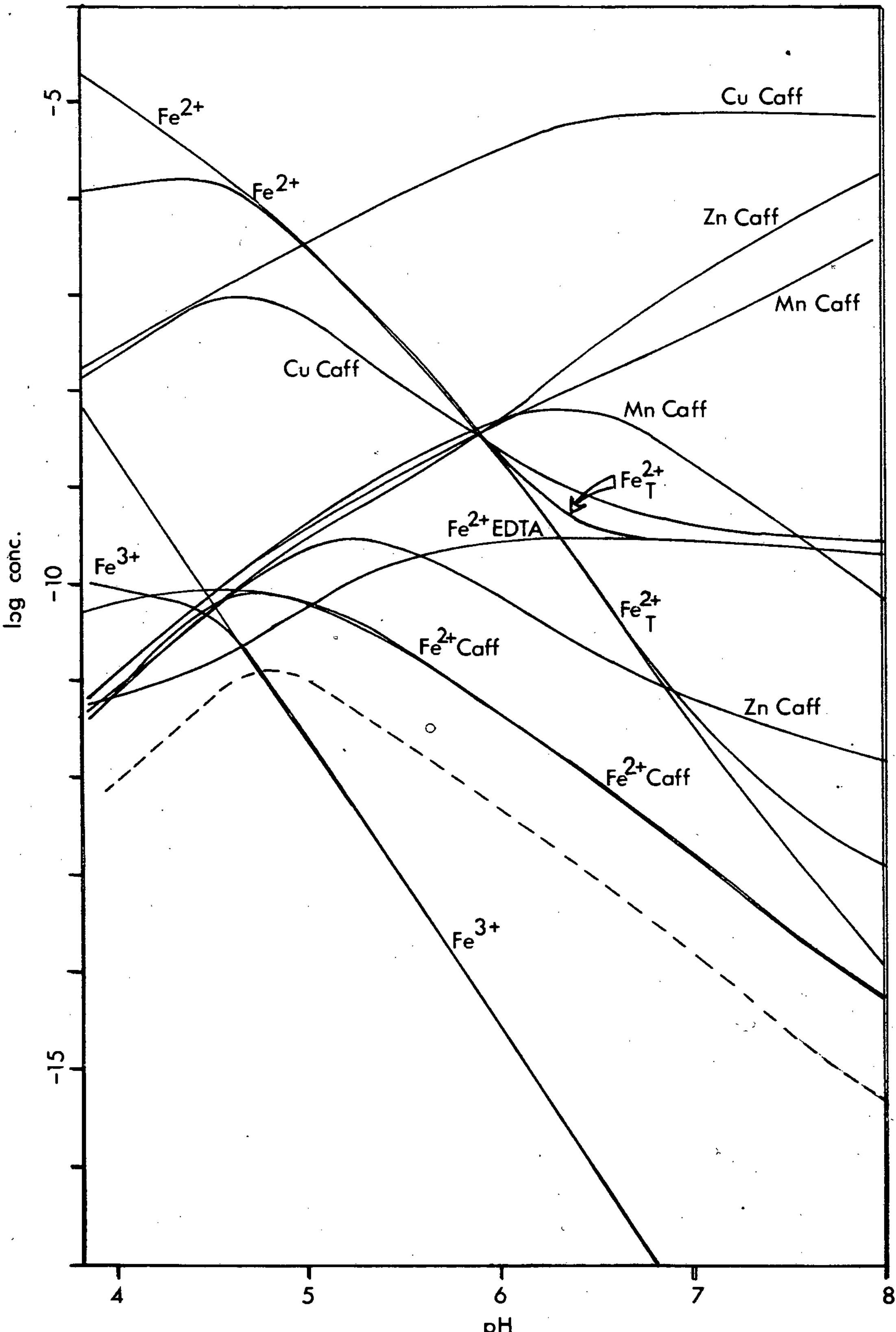
Modelling of solution B' was carried out in the absence of EDTA. The speciation results are presented in Figure 3.21 overlayed on Figure 3.20 for easier comparison. Figure 3.20 is identical to Figure 3.16, showing however a more complete set of speciation curves thereby giving an overall indication of the complexation effect of caffeic acid on micronutrient speciation in solution. From these it can be seen that EDTA has quite a marked effect on the speciation of the various caffeic acid complexes of manganese(II), copper(II) and zinc(II), especially at higher pH. However the speciation curve for the iron(II) caffeate complex does not appear to be altered by the presence of EDTA.

Furthermore, in the region of pH > 6, EDTA has little discernible influence on the free iron(II) concentration, as the respective curves of the two plots superimpose quite well. However, in this pH region the presence of EDTA increases the total iron(II) concentration significantly as is indicated by the large differences in the corresponding curves.This is probably due to the iron(II) EDTA complex. The free iron(II) and the total iron(II) curves in Figure 3.21, i.e. for the solution without EDTA, overlap for most of the pH region investigated. As the iron(II) caffeic complex is not very significant compared with the iron(II) free species, it can therefore not bring about a significant split as is observed when EDTA is present. Solution B' was modelled with a range of caffeic acid concentrations which resulted in varying total concentrations of all the iron(II) caffeate complexes, as expected. For instance, the dotted line in Figure 3.20 represents the iron(II) caffeic acid complexes on an 80% reduction of the caffeic acid concentration. However, no 'splitting' of the free and total iron(II) curves was observed

Figure 3.20 Speciation diagram of solution B' as for figure 3.16 but showing the additional complexes of caffeic acid with copper(II), zinc(II) and manganese(II). The dotted line represents Fe^{2+}Caff modelled in the presence of 20 micromol dm^{-3} caffeic acid.

Figure 3.21 Speciation diagram of solution B' modelled in the absence of EDTA.

See Appendix B for the abbreviations used in these figures.



even for 1000 micromol dm^{-3} caffeic acid concentrations.

Comparing the plots in the more acidic region, i.e. pH < 4.8, it can be seen that EDTA has the effect of decreasing the free iron(II) concentration. This is probably caused by a reduction in the iron due to EDTA complexation. It may be noted that a concurrent decrease in the free iron(III) concentration occurs. Therefore, the null hypothesis 3.6 stating that the effects observed of the inhibitors on the speciation, are not due to EDTA, can be rejected.

These results thus support our conclusions made in section 3.5.2.2 for the indirect inhibitory effect of manganese(II), copper(II) and zinc(II) observed at high pH, relatively well. However, for the low pH region the EDTA effect can only be a partial explanation for the unexpected increase in the iron(II) concentration upon the addition of the inhibitors in the presence of caffeic acid. This is because the EDTA effect is not nearly as great as that produced by the inhibitors in this region.

3.5.2.4 Discussion

The decreasing iron(II) and iron(III) concentrations with increasing pH observed in all the speciation plots may be attributed to the increased precipitation of iron(III) by the thermodynamically stable $Fe(OH)_3$ solid. This can account for the inhibitory effect of hydroxy ions on the reduction of caffeic acid as observed by Olsen et al (18). However this manifests itself also in the solutions without caffeic acid.

Although the inhibitory effect of phosphate was not specifically studied we propose that it manifests itself in the same way as OH^- inhibition, by precipitation of the $Fe(PO_4)$ solid. This is suggested on the basis of precipitation of this solid, observed in the preliminary modelling with the complete nutrient solution B.

As a matter of interest, it is observed that the presence of caffeic acid maintains a rather high iron(II) concentration even in the presence of the strong chelator, EDTA. This concentration is greater than 10^{-9} mol dm^{-3} throughout the acidic region of pH < 6. Together with EDTA, the total iron(II) concentration in the region of pH > 6 is larger than $10^{-9.5}$ mol dm^{-3}. The iron(II) concentration is therefore of the same order of magnitude for the whole pH range as as the minimum level required for normal growth of $10^{-9.2}$ mol dm^{-3}, quoted by Wallace (117) from the investigations carried out by Schwab. Lindsay and Schwab found that uptake at concentrations below this value was independant of the free iron(II) concentration.They propose that this reflects the uptake of complexed iron as has also been reported by other workers (125, 126). Lindsay and Schwab (14) however proposed a larger value of $10^{-7.7}$ mol dm^{-3} as being an adequate free iron concentration maintaining a level of 50 ppm of iron in the plants studied. From Figure 3.16, it can be seen that for most of fthe pH region < 6, the free iron(II) concentration is quite significantly larger than even this minimum value, and is in marked contrast to the solution modelled in the absence of caffeic acid. For plants grown in basic conditions the modelled free iron(II) concentration is considerably less even in the presence of caffeic acid, which suggests further mechanisms, than just the observed release of reducing substances into the surrounding solution to be operative.

From Figure 3.17 it can be seen that in the absence of caffeic acid the highest concentration of either of the iron aquo species is only just greater than 10^{-10} mol dm^{-3}. Furthermore this is the case over only a very small part of the acidic region i.e. for pH < 4.5. Using the nutrient solution modelled here in biological experiments with plants that do not exude caffeic acid or a similar ligand, this would be expected to result in iron deficiency. This argument is based on the assumption that one or the other of the iron aquo ions is the bioavailable form of this nutrient required by plants for normal growth.

From Figure 3.16 or 3.20 it can be clearly seen what a large effect the primary iron stress response mechanism of increasing the acidity of the solution, observed for iron efficient plants could have on the free iron(II) concentration in the presence of caffeic acid.

Such a marked effect is not evident in the solution without caffeic acid (Figure 3.17) and therefore the decrease in pH would not be so effective, resulting in only slightly elevated free iron concentrations as discussed above. Thus the speciation study can account quite well for the iron stress response mechanism observed in iron 'efficient' plant species on experiencing iron stress due to insufficient concentrations of this nutrient.

It has been proposed that the differences observed between iron 'efficient' and 'inefficient' plants is related to their ability to exude sufficient quantities of these reducing compounds. Thus any modelling which does not include reducing exudates may be too far removed from reality to be of significant use.

What now remains to be dicussed is the effect of redox on the speciation in solution. Schwab and Lindsay suggested that for solutions in the pH + pE range of 8 to 16, ferrosic hydroxide $Fe_3(OH)_8$ is the solid phase controlling free iron concentration.

The solutions containing caffeic acid modelled here had pH +

pE values of about 12 to 13 which are close to the redox requirements suggested by Lindsay and Schwab for adequate iron uptake. However, the only iron hydroxy precipitates ever observed in this study were the thermodynamically stable solids $Fe(OH)_3$ and in a few instances Fe_3O_4, i.e. magnetite. As the modelling study assumes thermodynamic equilibrium this was not unexpected because $Fe_3(OH)_8$ is a metastable solid and will with time become converted to the thermodynamically more stable iron oxides. Schwab and Lindsay also suggested that this conversion would eventially occur. They proposed as a reason for the existance of the metastable ferrosic hydroxide $Fe_3(OH)_8$, the dynamic nature of their solutions and those of other workers with respect to changing redox conditions. They proposed as a further reason for this observation the possible existance of reduced microsites in their soil suspensions studied (14).

The model is thus a 'longterm model' simulating aged solutions in thermodynamic equilibrium. This suggest that short term studies of fresh solutions should be carried out, thereby taking into account kinetic factors. These could for example be simulated by modelling in the absence of the $Fe(OH)_3$ and Fe_3O_4 precipitation equilibria. Thus further modelling was carried out. However, no iron solids were now observed to precipitate, not even ferrosic hydroxide as reported by Lindsay and Schwab. The free iron concentrations, as well as the concentrations of the various iron(II) complexes, however increased markedly. As Lindsay and Schwab suggest further studies are required in this direction.

The computed pH + pE values of the solutions without caffeic acid were found to be about 18 which is an increase of roughly 5 logarithmic units over that found for solutions with caffeic acid indicating lower iron(II) concentration. This decrease in reduction potential of the solution is therfore obviously closely linked to the absence of caffeic acid. This possibly causes a tendency towards greater precipitation of $Fe(OH)_3$ as is indicated by the modelling results showing only the presence of this solid. Due to its greater stability this would result in lower free iron concentrations.

Finally a comment pertinent to the lower limit of the concentration of soluble iron(II) proposed by Lindsay and Schwab should be made. In this modelling study a value for logK for the precipitation of $Fe(OH)_3$ by the reaction

$$Fe^{3+} + 3H_2O \rightleftharpoons Fe(OH)_3 + 3H^+$$

of -3.20 at 0.00 ionic strength was used, see discussion in section 3.3.2.1. This is rather large when compared to that used by Truesdell and Jones (79) and Nordstrom (61) of -4.9 in their modelling studies, using the program WATEQ2, and is closer to that determined by Schindler for the amorphous aged solid of -3.55 in 3.0 mol dm^{-3} sodium perchlorate calculated in this study to be -2.57 at 0.0 mol dm^{-3} ionic strength (89).

Thus, as occurred with the modelling in the absence of $Fe(OH)_3$, using the constant of -4.9 which is at the lower end of the range would again have resulted in higher iron(II) concentration. Redox control of hydroponic cultures might therefore be a useful means to avoid iron deficiency in plants. This is unknowingly practised by the flooding of rice fields, to the extent that iron toxicity frequently occurs, as is suggested by Jugsujinda and Patrick (127).

The speciation of the other micronutrients in the presence of caffeic acid is also of some interest. This is further discussed in Chapter 4, from a different point of view to that of speciation in solution. As can be seen from Figure 3.21 caffeic acid complexes nearly all of the copper(II) and most of the zinc(II) and manganese(II) at high pH in the absence of EDTA. However, this ligand does not nearly complex the micronutrients to the same extent as EDTA and is a result of the much lower stability constants of the former. Thus a fairly large free metal aquo ion concentrations is maintained by the caffeic acid, not shown here. As it has often been proposed that a particular micronutrient cation deficiency is the result of competition between the root and a strongly chelating ligand (123), adequate free ion concentration could be a condition for normal growth. This argument obviously depends largely upon the respective aquo ions being the bioavailable form of the micronutrients that are required by the plant. The importance of the manganese(II) aquo ion has recently been proposed for manganese nutrition by Schwab and Lindsay (122). The particular bioavailable form of a nutrient required is however not always completely clear as in the case of zinc (121, 116). It is therefore suggested that caffeic acid could be used as an alternative complexing ligand in hydroponic cultures especially for acidic conditions.

CHAPTER FOUR

GENERAL DISCUSSION

In this chapter some ideas on the uptake of micronutrients and nutrition of plants will be proposed. Due to the great diversity of plant nutrition only a few aspects will be discussed here. Various interpretations and conclusions are made, based on the literature and the results of this study reported in sections 2.4 and 3.5. Furthermore the extent to which this study has been successful in meeting the objectives set out in Chapter 1, will be discussed. Thus also some areas of possible future research that have arisen during the course of this study will be pointed out.

First the potentiometric results will be discussed. The investigation of the protonation equilibria lead to the determination of two formation constants of the triprotic caffeic acid ligand. These compared very favourably with the values reported by Timberlake (24) for slightly different ionic strengths. It is suggested that deprotonation of the third hydrogen ion occurs only in the presence of metal ions or in very basic conditions as this third proton is strongly held by hydrogen bonding.

Four of the proposed five caffeic acid metal ion systems, i.e. copper(II), zinc(II), iron(III) and iron(II) were investigated. Due to rapid and virtually complete reduction of iron(III) by the caffeic acid only three of these could be succesfully characterized by the method of potentiometry. Caffeic acid is an interesting ligand in its own right from an inorganic point of view as it possesses complexation sites at either end of the molecule and can thus act as a bridging ligand. This therefore leads to the proposal of some interesting structures especially on complexation with copper(II) ions, for which polynuclear species are indicated. Similar polynuclear speciation had been reported by Fazakerley et al in their studies of the copper(II)-methyldopa system (56) and is fairly typical of copper(II) complexing behaviour with ligands that have more than one coordination site.

Both the copper(II)- and zinc(II)-caffeic acid systems were characterized quite well. This is indicated by the relatively small R values and the very good superimposability obtained between theoretical and experimental formation curves. See Figures 2.6 and 2.7 for copper(II) and Figures 2.9 and 2.10 for zinc(II). Slightly less accurate results were obtained for the system with iron(II) but this is a notoriously difficult metal ion to deal with experimentally (59). For all three systems the technique of pseudoplotting (39) was extensively used as it was found to be a very sensitive method of distinguishing between alternative hypotheses.

Potentiometry can be used only to determine the stoicheiometry of complexes and not to elucidate the actual structures or the sites of metal binding. However, the approach of comparison with literature constants for ligands having suitably similar coordination sites to those of caffeic acid, was used to infere actual structures for some of the complexes found. These need to be verified by an alternative experimental technique.

The three speciation diagrams, Figures 2.8, 2.11 and 2.14 for copper(II), zinc(II) and iron(II) respectively, give an indication of the effect of caffeic acid complexation of these metal ions in plant nutrition. Although the constants determined are not particularly large by comparison with those for some of the synthetic chelates used in nutrient solutions, it is seen from these diagrams that caffeic acid complexes the metal ions of copper(II), zinc(II) and to a lesser extent iron(II) reasonably strongly. This results in virtually all of the metal ion present being complexed by caffeic acid at about pH 6 and 7.5 for copper(II) and zinc(II) respectively, while most of the iron(II) aquo ion is complexed at a pH of 8. The extent of complexation is also indicated by the respective formation curves, all of which show large Z values.

The constants for manganese(II) with caffeic acid were only estimated and therefore still need to be experimentally determined in future research. These might be of significant importance as Tyson and Martell have pointed out that catechol forms one of the most stable complexes of a bidentate ligand with manganese (52).

A major aspect of this thesis comprises the construction of a relatively extensive plant nutrient model, using constants reported in the open literature as well as the caffeic acid complexation constants reported in Chapter 2. The literature constants were adjusted where necessary for ionic strength using an extended form of the Debye-Hückel equation.

It was found necessary to consider various kinetic factors pertinent to the nutrient solution conditions which were thus incorporated into the model. These are involved with the distribution of redox species by the H_2O/O_2 redox couple of dissolved oxygen, and the dissolution of solid calcium carbonate which is frequently used in hydroponic plant experiments to simulate calcareous soil conditions with high pH. The redox distribution problem was solved relatively easily by simply omitting the oxygen redox couple from the model and allowing the redox species to distribute amongst their oxidation states. This was facilitated by the introduction of caffeic acid. The justification for the forgoing approximation was based largely on the findings of Ball et al (91), who suggest that the H_2O/O_2 couple which is usually incorporated in modelling studies, is kinetically inert. We propose that the reduction potentials of nutrient solutions maintained by exuded reductants or microbial action are far greater than those maintained by oxygen even under anaerobic conditions.

The carbonate problem for systems at low carbon dioxide partial pressures together with slow calcite dissolution rates has been studied extensively by a number of workers (110, 111). Although the studies of Plummer and co-workers represent an excellent investigation of calcite dissolution, this did not lead to a very successful explanation or a strictly correct solution of the problems with respect to pH as encountered in this study. However they have shown that rates of dissolution are quite slow especially at low carbon dioxide partial pressures. Thus by an empirically determined adjustment of one of the relevant stability constants the problem was solved satisfactorily for the purposes of this study. This is not to say that other kinetic factors might not play a part in the pH discrepancies observed or that other reasons might not be important. Thus various other constants were considered for adjustment, which were those of the various iron hydroxy solids, with particular reference also to the redox speciation, and the calcium hydroxy apatite solubility product. The need for further studies of kinetic factors seems to be indicated.

Even though many different systems were used for validation of the model, it is felt that further validation is probably neccessary. Validation by comparison of computed with measured pH turned out to be adequate for the purposes of this study, but a better test of the model is desirable. This is due to pH being governed largely by macronutrients and it is thus difficult to validate the micronutrient concentrations. Possibly, redox measurements with a platimum electrode could be carried out on the nutrient solutions to validate the computation of the redox species. However this is fraught with problems as indicated in section 3.4.4. A precipitation study could be carried out in which the nutrient solution is reduced in volume and the solids that precipitate are characterized and compared with those for which conditions of supersaturation exist in the modelled solutions. This was carried out in a study on the modelling of urine (74). However as the volume of the experimental solution is reduced, ionic strength increases and various other assumptions are required to be made, which might cause the results to be questionable. Other ion sensitive electrodes could be used in conjunction with pH measurements and a titration with mineral acid or base performed on the nutrient solution. This is thought to possibly represent the best method of validation of those proposed as a number of free ion concentrations could be determined and compared with computed values at various discrete pH's. However due to the complexity of the nutrient solution interfering ions could present some problems with these measurements.

In the modelling section on the chemical speciation of nutrient solutions two important problems in the nutrition of plants were relatively successfully investigated. These are namely the effect of chelation and the effect of plant exudates on micronutrient speciation in solution and hence their effect on plant nutrition. In the first section (3.5.1), i.e. on the effect of micronutrient chelation, some anomalies are apparent in the literature.

Results of experiments conducted in soils do not often correlate well with those obtained from nutrient solutions (3, 116, 120). It has been proposed (3) that essentially chelation in soils is necessary to mobilise the various micronutrients and maintain adequate levels of water soluble species of these in the soil solution bathing the roots. This is because most of the micronutrients, for example copper and manganese and particularly iron, are bound by their respective hydroxy solids in soils and are therefore largely unavailable to the plants. The ligands therefore provide the carrier mechanism by which depleted nutrients at the root surface are replenished by increasing both the mass-flow and diffusion rates to the roots. On the other hand, uptake of nutrients from hydroponic solutions has been correlated with the free aquo ion concentrations, resulting in the chelate inhibition theory (123, 5, 118) theory which is rationalized by the results of this study, section 3.5.1 and the modelling results of Halvorson and Lindsay (121). This represents a severe discrepancy with the results found in soils. Lindsay (3) explains this on the basis of a build up of chelator on dissociation and uptake of the nutrient by the plant in solutions, whereas in soils the ligand can diffuse away and get saturated with, for instance, more iron from the oxides present.

Another anomaly however appears in the literature on experimental results of plants grown in nutrient solutions. In some instances, iron uptake from solution is increased by the use of the strong chelator EDDHA and even by small excesses of this and other ligands such as EDTA and DTPA (108, 124). In some instances it has been reported that uptake is not adversely affected even by large excesses of EDDHA (118, 119, 117). Both of the above observations, which indicate that chelation facilitates uptake of metal ions from nutrient solutions are in marked contrast to the chelate inhibition theory. Generally for solutions modelled at low pH in this study ligands did not seem to be required as sufficient concentrations of soluble species, in particular those of the free metal ions, were present. When large concentrations of phosphate were present, the concentration of iron soluble species was decreased by the precipitation of iron phosphate, however. Similarly at higher pH, i.e. greater than 6, a considerable proportion of the iron is precipitated to some extent by phosphate, depending on the composition of the solution, but always by hydroxy ions to form the thermodynamically stable iron oxide, $Fe(OH)_3$. This results in greatly decreased concentrations of both iron(II) and iron(III) soluble species even in the presence of ligands at high pH of about 7.5 to 8.0. This has also been shown by the use of stability diagrams for the speciation of iron in soils in various studies (130, 83). The other micronutrients by contrast to the conditions present in soil were generally not precipitated by their oxides for the nutrient compositions as modelled in this study. Thus , based on these considerations alone, it is suggested that these nutrients do possibly not require ligands to be present in solution other than to avoid toxicity caused by excessive free aquo ion concentrations (120). However, as ligands are required at least for iron nutrition, this obviously complicates the overall nutrition by plants tremendously, because iron is not selectively chelated.

Linehan and Shephard (124) have reported that hydrolysis of iron(III) salts results in rapid formation of large fairly stable polymer species (131). In the absence of ligands the rate of iron uptake depends on the concentration of the free metal aquo ions in solution. However, this depends in turn on the rate of dissociation of the hydrolysis products which is possibly more important. As it is suggested that the rate of dissociation of these large polymeric iron species is relatively slow this would limit iron uptake from nutrient solutions (124). Adding ligands would only reduce the free aquo ion concentrations, but would increase the iron chelate concentration. The iron chelate is thought to be capable of rapid dissociation replacing the iron taken up by the plants. Thus Linehan and Shephard propose that optimal conditions would result with increased ligand concentrations such that all iron is complexed. Increasing the ligand concentration further, would result in reduced dissociation of the iron complex and decreased iron uptake. As the chelate is bound by various other cations in solutions, larger concentrations than expected are required to complex all the iron. This explains the apparent anomaly of increased uptake of iron with increased ligand concentration very well, as it is customary to add an amount of ligand equal to the iron concentration. Thus from this it is evident that various other metal ions for example calcium(II) can have a marked effect on the iron nutrition as these can effectively modify the solution conditions. Many plants thus exhibit various mechanisms to overcome iron deficiency by competing successfully with the ligands in a thermodynamic sense for the chelated iron. These represent the so-called iron stress response mechanisms and are discussed in the rest of this section, on the effects of plant exudates.

From the above it can be seen that iron nutrition is rather complex and at the same time of considerable importance. Two hypotheses have been proposed by workers in the field of plant nutrition with respect to the mechanism of reduction of iron(III) to iron(II). These hypotheses have been stated in section 2.1. The modelling results of this study have shown that caffeic acid has a significant effect on the speciation of iron in solution, greatly increasing the iron(II) aquo ion concentration, see Figures 3.16 and 3.17. This represents strong evidence in support of the hypothesis of Brown and co-workers in section 2.1. This states that plants exude reducing substances into solution in order to increase reduction and hence uptake of iron, upon experiencing conditions of iron deficiency stress. This is also supported by the qualitative results on the investigation of the iron(III) caffeic acid system, section 2.4.2.3. This does not neccessarily mean, however, that we express ourselves in favour of the hypothesis of Brown and co-workers. It has often been suggested, even by Brown himself (18), that the observed release of reductants might just be due to a 'leaky' root system, resulting from release of hydrogen ions on iron stress. Even if this were the case and release of reducing substances were only a result of the stress response mechanism, the effect on solution speciation of caffeic acid could not be ignored. Thus any modelling study, especially of iron nutrition, should take into account exuded reductants. Modelling in their absence could lead to speciation results that are too far removed from reality to be of any use. Furthermore, this was also a useful means of distributing the redox species amongst their oxidation states. This should however be experimentally verified in the future.

The speciation results obtained in the present work could not be used to explain the observed effects of the 'inhibitors' manganese(II), zinc(II) and copper(II) very well. In fact, an increase in the free iron(II) concentration in the presence of inhibitors is suggested by Figures 3.16 and 3.19 in the region of low pH. This was further investigated in section 3.5.2.3 on the effects of EDTA on iron speciation in the presence of caffeic acid. Thus the inhibitory effect observed in plant experiments (128) is probably not primarily due to speciation changes in solution. The results therefore suggest noncompetitive inhibition, with iron reduction taking place at specific sites on the root membrane, possibly by an enzymatic process. Thus the second hypothesis in section 2.1, stating that reduction of iron is an enzymatic membrane bound process occurring at specific sites on the roots is supported by the modelling results of sections 3.5.2.2. and 3.5.2.3.

Much experimental evidence by a number of different workers has recently been reported which supports the second hypothesis stated in section 2.1 (19, 20, 21, 12). These suggest that increased iron uptake is due to increased chelate splitting ability which is associated with reduction at the plasmalemma by plants of the iron efficient variety during conditions of iron stress. Various physiological changes are observed to occur in the structure of the plant roots. Marschner et al (22) have in this respect reported observing a marked build up of phenolics in the root tips that are affected. They suggest that these might be involved with the obligatory reduction of iron(III) to iron(II). This in itself constitutes increased chelate splitting ability as for most ligands used in nutrient solutions the formation constants for iron(II) are considerably smaller than for iron(III). Thus caffeic acid could quite conceivably be involved in the enzymatic reduction of iron(III), in this case acting as a carrier in the transport of metal ions across the plasmalemma. This requires a slight shift in the approach to the speciation results. Whereas generally these have been viewed with the chelate inhibition hypothesis in mind, correlating free aquo ion concentration with uptake by plants, the total concentrations of chelated species now becomes of interest. Considering the caffeic acid to be a membrane bound carrier, the fairly pronounced complexation of manganese(II), zinc(II) and copper(II) with this ligand, observed in Figures 3.20 and 3.21, could well account for the increased uptake of these nutrients under iron stress conditions observed by Röheld et al (129). This could also account for the anomaly in the study of excess chelating agents on micronutrient concentrations, found for EDTA at high pH, section 3.5.1.2.

Thus by not considering exudates confusing trends can become evident. This applies to much of the literature on the study of metal uptake by plants. What obviously contributes greatly to this, is the fact that plants react differently from one to another, especially when considering different species as they can modify the solutions in which they grow. By considering exudates in the modelling of factors affecting nutrient uptake by plants some of these variations have been taken into account in this study.

The study has thus, following the systematic approach to modelling developed in section 3.1, accomplished most of the aims listed in Chapter 1 with varying degrees of success. Furthermore, the model has hopefully contributed to the understanding of a few aspects involved in the nutrition of plants.

APPENDIX A

Database of the equilibrium constants of the complex species taken into account in the chemical modelling of plant nutrient solutions in this study. These are given in the form of the input required by the computer program MINEQL. The table below gives the various component numbers used.

Component number	Chemical abbreviation[#]	Component number	Chemical abbreviation[#]
1	Ca^{2+}	101	$CO_3{}^{2-}$
2	Mg^{2+}	102	$SO_4{}^{2-}$
4	K^+	103	CL^-
5	Na^+	107	NH_3
6	Fe^{3+}	109	$PO_4{}^{3-}$
7	Fe^{2+}	117	Cit^{-3}
8	Mn^{2+}	128	$EDTA^{4-}$
9	Cu^{2+}	148	$B(OH)_4{}^-$
12	Zn^{2+}	152	$MoO_4{}^{2-}$
33	Cu^+	157	$NO_3{}^-$
34	Mn^{3+}	159	$DTPA^{5-}$
50	H^+	160	$EDDHA^{4-}$
99	e^-	161	$Caff^{2-}$
		162	[a]Quin

[#] The chemical symbols have their normal elemental meanings. See text for the abbreviations used for the ligands.

[a] Quin represents the oxidation product of caffeic acid, i.e. caffeoyl-o-quinone

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00299
70000     2.31   1  1 102  1
70002     6.46   1  1 109  1
70004    14.75   1  1 109  1   50   1
70006    20.54   1  1 109  1   50   2
70008    28.44   1  1 109  2   50   2
70010    33.75   1  1 109  2   50   3
70012    30.06   1  2 109  2   50   2
70020    12.28   1  1 128  1
70022    15.79   1  1 128  1   50   1
70024   -12.70   1  1  50 -1
70030     3.15   1  1 101  1
70032    11.33   1  1 101  1   50   1
70034    -0.29   1  1 107  1
70036    -1.09   1  1 107  2
70038    -2.09   1  1 107  3
70026     0.70   1  1 157  1
70028     0.60   1  1 157  2
71706     4.02   1  1 117  1
71708     8.69   1  1 117  1   50   1
71710    11.46   1  1 117  1   50   2
75907    12.40   1  1 159  1
75918    18.13   1  1  50  1  159   1
75908    15.15   1  2 159  1
76005     8.57   1  1 160  1
76006    18.20   1  1  50  1  160   1
76007    26.08   1  1  50  2  160   1
70100     2.23   2  1 102  1
70102     6.59   2  1 109  1
70104    15.25   2  1 109  1   50   1
70106    21.05   2  1 109  1   50   2
70108    34.23   2  1 109  2   50   3
70110    28.41   2  1 109  2   50   2
70112    30.64   2  2 109  2   50   2
70114    10.05   2  1 128  1
70116    14.37   2  1 128  1   50   1
70118   -11.42   2  1  50 -1
70120   -39.70   2  4  50 -4
70122     3.90   2  1 101  1
70124    11.88   2  1 101  1   50   1
70126     0.80   2  1 107  1
70128    -0.27   2  1 107  2
70130    -0.85   2  1 107  3
70132    -1.75   2  1 107  4
71702     4.62   2  1 117  1
71704     8.97   2  1 117  1   50   1
76008     9.36   2  1 160  1
76009    18.59   2  1  50  1  160   1
76010    26.71   2  1  50  2  160   1
75913    10.89   2  1 159  1
71744     4.32   2  1 117  2
70900   -14.50   4  1  50 -1
70904     0.80   4  1 102  1
70908    13.43   4  1 109  1   50   1
70910    -0.76   4  1 103  1
70902    -0.15   4  1 157  1
71700     1.26   4  1 117  1
70200     0.70   5  1 102  1
70202     1.51   5  1 102  2
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70204	2.28	5	1	128	1		
70208	13.54	5	1	109	1	50	1
70210	2.13	5	1	148	1		
70212	-14.20	5	1	50	-1		
70206	-0.51	5	1	157	1		
70400	4.04	6	1	102	1		
70402	5.38	6	1	102	2		
70404	1.48	6	1	103	1		
70406	2.13	6	1	103	2		
70408	1.13	6	1	103	3		
70412	22.44	6	1	109	1	50	1
70414	23.53	6	1	109	1	50	2
70415	29.24	6	2	109	1	50	1
70416	26.97	6	1	128	1		
70418	28.34	6	1	128	1	50	1
70450	26.25	6	1	128	1	50	2
70420	19.22	6	1	128	1	50	-1
70422	9.44	6	1	128	1	50	-2
70434	-3.49	6	1	128	1	50	-3
70424	9.05	6	1	148	1		
70426	16.41	6	1	148	2		
70428	21.50	6	1	148	3		
70430	8.73	6	1	152	1		
70432	17.33	6	1	152	3		
70436	-2.19	6	1	50	-1		
70438	-5.70	6	1	50	-2		
70440	-9.51	6	1	50	-3		
70442	-21.60	6	1	50	-4		
70444	-2.90	6	2	50	-2		
70446	-6.30	6	3	50	-4		
70448	1.00	6	1	157	1		
71720	12.25	6	1	117	1		
71722	16.56	6	1	117	2		
75911	31.02	6	1	159	1		
75912	34.94	6	1	159	1	50	1
76018	35.87	6	1	160	1		
70500	2.20	7	1	102	1		
70502	2.70	7	1	102	1	50	1
70504	-9.50	7	1	50	-1		
70506	-20.60	7	1	50	-2		
70508	-32.00	7	1	50	-3		
70510	-46.40	7	1	50	-4		
70512	15.62	7	1	128	1		
70514	18.67	7	1	128	1	50	1
70516	21.78	7	1	128	1	50	2
70518	6.21	7	1	128	1	50	-1
70520	-4.28	7	1	128	1	50	-2
70522	15.94	7	1	109	1	50	1
70524	22.24	7	1	109	1	50	2
70526	29.52	7	1	109	2	50	2
71716	4.97	7	1	117	1		
71718	9.08	7	1	117	1	50	1
77070	4.64	7	1	161	1		
77071	7.46	7	2	161	1		
77072	-3.29	7	1	161	1	50	-1
77073	-.19	7	1	161	2	50	-1
77074	-7.69	7	1	161	3	50	-2
75905	17.98	7	1	159	1		
75906	23.80	7	1	159	1	50	1

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76019     15.61   7  1 160  1
70804     15.91   8  1 109  1   50   1
70806     22.24   8  1 109  1   50   2
70808     29.27   8  1 109  2   50   2
70810      2.26   8  1 102  1
70812    -10.60   8  1  50 -1
70814    -22.31   8  1  50 -2
70816    -34.20   8  1  50 -3
70818    -48.30   8  1  50 -4
70820    -10.60   8  2  50 -1
70822    -23.90   8  2  50 -3
70923     12.13   8  1  50  1  101   1
70824     15.24   8  1 128  1
70826     18.69   8  1 128  1   50   1
70828     21.71   8  1 128  1   50   2
70832      0.86   8  1 107  1
70834      1.49   8  1 107  2
70836      1.75   8  1 107  3
70838      1.32   8  1 107  4
70800      0.20   8  1 157  1
70802      0.60   8  1 157  2
71712      4.46   8  1 117  1
71714      8.64   8  1 117  1   50   1
77080      3.98   8  1 161  1
77081     -4.46   8  1 161  1   50  -1
77082    -13.09   8  1 161  2   50  -2
75914     17.13   8  1 159  1
71746      4.62   8  1 117  2
70704     16.35   9  1 109  1   50   1
70706     21.06   9  1 109  1   50   2
70708     33.44   9  1 109  2   50   3
70710     33.08   9  2 109  2   50   2
70712     29.81   9  1 109  2   50   2
70714      2.36   9  1 102  1
70716      3.76   9  1 102  2
70718      2.55   9  1 102  3
70720      7.53   9  1 148  1
70722     13.08   9  1 148  2
70724     15.81   9  1 148  3
70726     -7.70   9  1  50 -1
70728    -14.80   9  1  50 -2
70730    -27.58   9  1  50 -3
70732    -39.60   9  1  50 -4
70734    -10.90   9  2  50 -2
70736    -21.60   9  3  50 -4
70738    -20.90   9  4  50 -3
70740     20.05   9  1 128  1
70742     23.38   9  1 128  1   50   1
70744     25.01   9  1 128  1   50   2
70746     23.77   9  1 128  1   50   3
70748      8.05   9  1 128  1   50  -1
70750      6.75   9  1 101  1
70752      9.83   9  1 101  2
70754      0.40   9  1 103  1
70756      4.06   9  1 107  1
70758      7.36   9  1 107  2
70760     10.10   9  1 107  3
70762     11.99   9  1 107  4
70764     11.43   9  1 107  5
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70766     -2.80   9  1 107  1   50 -1
70768     -0.80   9  1 107  3   50 -1
70770    -15.70   9  1 107  2   50 -2
70700      0.50   9  1 157  1
70702      0.42   9  1 157  1
71724      6.47   9  1 117  1
71726      9.85   9  1 117  1   50  1
71728     13.03   9  1 117  1   50  2
71730      9.16   9  1 117  2
71732      2.95   9  2 117  1
71734     14.26   9  2 117  2
77090      6.80   9  1 161  1
77091     11.21   9  1 161  1   50  1
77092       .79   9  1 161  1   50 -1
77093      4.47   9  2 161  1   50 -1
77094       .44   9  2 161  3   50 -3
77095      8.89   9  3 161  2   50 -2
76011     25.25   9  1 160  1
76012     33.64   9  1  50  1  160  1
76013     38.79   9  1  50  2  160  1
75909     23.03   9  1 159  1
75910     27.80   9  1 159  1   50  1
71204     15.55  12  1 109  1   50  1
71206     21.12  12  1 109  1   50  2
71208     34.40  12  1 109  2   50  3
71210     31.65  12  2 109  2   50  2
71212     29.21  12  1 109  2   50  2
71214      2.38  12  1 102  1
71216      3.72  12  1 102  2
71218      3.17  12  1 102  3
71220      2.38  12  1 102  4
71222     -9.00  12  1  50 -1
71224    -15.08  12  1  50 -2
71226    -28.40  12  1  50 -3
71228    -41.20  12  1  50 -4
71230     -9.00  12  2  50 -1
71232     18.39  12  1 128  1
71234     21.74  12  1 128  1   50  1
71236     22.91  12  1 128  1   50  2
71238      6.18  12  1 128  1   50 -1
71242      4.70  12  1 101  1
71243     12.13  12  1  50  1  101  1
71244      0.43  12  1 103  1
71246      1.04  12  1 103  2
71248      1.54  12  1 103  3
71250      1.74  12  1 103  4
71252      2.21  12  1 107  1
71254      4.50  12  1 107  2
71256      6.86  12  1 107  3
71258      8.89  12  1 107  4
71260     -4.39  12  1 107  1   50 -1
71262     -2.83  12  1 107  2   50 -1
71264     -1.64  12  1 107  3   50 -1
71266    -14.36  12  1 107  1   50 -2
71268    -13.77  12  1 107  2   50 -2
71270    -26.92  12  1 107  1   50 -3
71200      0.40  12  1 157  1
71202      0.64  12  1 157  2
71736      4.33  12  1 117  1
```

71738	6.15	12	1	117	2		
71740	9.11	12	1	117	1	50	1
71742	12.02	12	1	117	1	50	2
75916	20.32	12	1	159	1		
75917	25.08	12	1	50	1	159	1
75919	25.80	12	2	159	1		
76015	26.24	12	1	50	1	160	1
76016	33.05	12	1	50	2	160	1
77120	3.78	12	1	161	1		
77121	-2.48	12	1	161	1	50	-1
77122	-.20	12	1	161	2	50	-1
77123	-8.63	12	1	161	2	50	-2
77124	-6.98	12	1	161	3	50	-2
73300	10.18	33	1	107	2		
73400	3.44	34	1	102	1		
73402	0.81	34	1	50	-1		
73404	27.38	34	1	128	1		
73406	30.25	34	1	128	1	50	1
75915	21.77	34	1	159	1		
70600	10.33	50	1	101	1		
70602	16.68	50	2	101	1		
70604	1.99	50	1	102	1		
70606	-5.40	50	2	102	1		
70610	12.34	50	1	109	1		
70612	19.54	50	2	109	1		
70614	21.68	50	3	109	1		
70616	30.79	50	3	109	2		
70618	38.75	50	4	109	2		
70620	41.77	50	5	109	2		
70622	10.91	50	1	128	1		
70624	17.55	50	2	128	1		
70626	20.69	50	3	128	1		
70628	22.96	50	4	128	1		
70630	24.45	50	5	128	1		
70632	24.00	50	6	128	1		
70634	-14.00	50	-1				
70636	9.34	50	1	148	1		
70638	9.94	50	1	148	3		
70640	20.02	50	2	148	3		
70642	20.44	50	2	148	4		
70644	29.96	50	3	148	4		
70646	38.92	50	4	148	5		
70648	4.04	50	1	152	1		
70650	8.03	50	2	152	1		
70652	8.89	50	3	152	1		
70654	-6.20	50	1	103	1		
70656	9.24	50	1	107	1		
70658	6.40	50	1	117	1		
70660	11.16	50	2	117	1		
70662	14.28	50	3	117	1		
75500	10.74	50	1	107	1	103	1
75502	10.35	50	1	107	1	102	1
75900	11.31	50	1	159	1		
75901	21.15	50	2	159	1		
75902	27.25	50	3	159	1		
75903	31.95	50	4	159	1		
75904	36.15	50	5	159	1		
70608	-1.42	50	1	157	1		
76000	12.25	50	1	160	1		

```
76001   23.01  50  2 160  1
76002   32.01  50  3 160  1
76003   38.51  50  4 160  1
77000    9.08  50  1 161  1
77001   13.67  50  2 161  1
00005
80000   13.02   6  1   7 -1  99  1
80002   25.53  34  1   8 -1  99  1
80004    2.68   9  1  33 -1  99  1
80008   13.68 162  1 161 -1  99  2
80010   21.65  50  2 101  1
00000
00039
80100    4.60   1  1 102  1
80102   43.75   1  5 109  3  50 -1
80104   46.90   1  4 109  3  50  1
80106   19.24   1  1 109  1  50  1
80108    8.36   1  1 152  1
80110  -22.97   1  1  50 -2
80112   28.92   1  3 109  2
80114   18.94   1  1 109  1  50  1
80116    8.49   1  1 101  1
80200   25.20   2  3 109  2
80202   18.16   2  1 109  1  50  1
80204  -16.85   2  1  50 -2
80206    8.09   2  1 101  1
80208    4.67   2  1 101  1
80210   17.00   2  1   1  1 101  2
80211   29.97   2  3   1  1 101  4
80212   21.35   2  1 107  1 109  1  50  1
80214   10.62   2  1   4  1 109  1
80600   -3.20   6  1  50 -3
80602   26.40   6  1 109  1
80606  -17.67   6  2   7  1  50 -8
80608   -9.60   6  2   7  1  50 -8
80700  -12.90   7  1  50 -2
80702   10.24   7  1 101  1
80704   36.00   7  3 109  2
80701    2.46   7  1 102  1
80800  -15.20   8  1  50 -2
80802    9.30   8  1 101  1
80900   -8.68   9  1  50 -2
80904   37.70   9  3 109  2
80906    9.63   9  1 101  1
80908    5.16   9  2 101  1  50 -2
80910   16.88   9  3 101  2  50 -2
80912   15.12   9  1 148  2
81200   35.30  12  3 109  2
81202   10.80  12  1 101  1
83302    6.73  33  1 103  1
83304  -13.30  33  1  50 -1
81204  -12.48  12  1  50 -2
00000
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APPENDIX B

Explanation of abbreviations used in Figures 3.16 to 3.21

Abbreviation	Meaning
Fe^{3+}	free iron(III) aquo ion
Fe^{2+}	free iron(II) aquo ion
$Fe^{2+}{}_T$	total iron(II) present in solution excluding iron(II) precipitates
Fe^{2+}EDTA	all iron(II) EDTA complexes
Fe^{2+}Caff	all iron(II) caffeic acid complexes
CuCaff	all copper(II) caffeic acid complexes
ZnCaff	all zinc(II) caffeic acid complexes
MnCaff	all manganese(II) caffeic acid complexes
Caff	caffeic acid^{2-} referring to the dianionic species, HL^{2-}
EDTA	ethylenediaminetetraacetic acid^{4-}, referring to the ligand species, L^{4-}

REFERENCES

1. D.P.Moore in " Micronutrients in Agriculture ", J.J.Mortvedt, P.M.Giordano and W.L.Lindsay Eds., Soil Sci.Soc.Amer., 1972.
2. H.F.Wilkinson in " Micronutrients in Agriculture ", J.J.Mortvedt, P.M.Giordano and W.L.Lindsay Eds., Soil Sci.Soc.Amer., 1972.
3. W.L.Lindsay in " The PLant Root and its Environment ", E.W.Carson Ed., University Press of Virginia, Charlottesville, 1974.
4. N.Ingri, W.Kakolowicz, L.G.Sillén and Bjorn Warnquist, Talanta, 1967, 14, 1261.
5. A.D.Halvorson, Ph.D. Thesis, Colorado State University, 1971.
6. E.A.Jenne, Chapter 1 in " Chemical Modeling in Aqueous Systems ", E.A.Jenne Ed., ACS Symposium Series, 1978.
7. J.C.Brown and J.E.Ambler, Agro.J., 1973, 65, 311.
8. R.A.Olsen and J.C.Brown, J.Pl.Nutr., 1980, 2(6), 629.
9. R.A.Olsen, J.C.Brown, J.H.Bennett and D.Blume, J.Pl.Nutr., 1982, 5(4-7), 433.
10. V.Römheld and H.Marschner, Plant Physiol., 1983, 71, 949.
11. R.A.Olsen, J.H.Bennett, D.Blume and J.C.Brown, J.Pl.Nutr., 1981, 3(6), 905.
12. E.G.Barrett-Lennard, H.Marschner and V.Römheld, Plant Physiol., 1983, 73, 893.
13. K.Venkat Raju, H.Marschner and V.Römheld, Z.Pflanzenernähr.Bodenkd, 1972, 132, 3, 177.
14. W.L.Lindsay and A.P.Schwab, J.Pl.Nutr., 1982, 5(4-7), 821.
15. J.C.Brown, Plant, Cell and Environ., 1978, 1, 249.
16. R.L.Chaney, J.C.Brown and L.O.Tiffen, Plant Physiol., 1972, 50, 208.
17. J.C.Brown and J.E.Ambler, Physiol. Plant., 1974, 31, 221.

18. R.A.Olsen and J.C.Brown, J.Pl.Nutr., 1980, 2(6), 647.

19. H.F.Bienfait, A.M. van der Bliek and R.J.Bino, J.Pl.Nutr., 1982, 5(4-7), 447.

20. H.F.Bienfait, J.Duivenvoorden and W.Verkerke, J.Pl.Nutr., 1982, 5(4-7), 451.

21. C.L.Tipton and J.Thowsen, Iowa State Journal of Research, 1983, 57(4), 409.

22. V.Römheld and H.Marschner, J.Pl.Nutr., 1981, 3(1-4), 551.

23. G.Julian, H.J.Cameron and R.A.Olsen, J.Pl.Nutr., 1983, 6(2), 163.

24. C.F.Timberlake, J.Chem.Soc., 1959, 2795.

25. L.Horner and E.Geyer, Chem.Ber., 1965, 98, 2016.

26. H.Rossotti, " The Study of Ionic Equilibria ", Longmans, New York, 1978.

27. K.Murray and P.W.Linder, J.Soil Sci., 1984, 35, 217.

28. P.W.Linder and K.Murray, Talanta, 1982, 29, 377.

29. M.T.Beck, " The Chemistry of Complex Equilibria ", Van Nostrand Reinhold, London, 1970.

30. F.R.Hartley, C.Burgess and R.Alcock, " Solution Equilibria ", Ellis Horwood, Chichester, 1980.

31. W.A.E.McBryde, Can.J.Chem., 1973, 51, 3572.

32. F.J.C. Rossotti and H.Rossotti, " The Determination of Stability Constants ", McGraw-Hill, New York, 1961.

33. P.W.Linder, R.G.Torrington and D.R.Wiliams, " Analysis using Glass Electrodes ", Open University Press, Belfast, 1984.

34. P.M.May, D.R.Williams, P.W.Linder and R.G.Torrington, Talanta, 1982, 29, 249.

35. A.Sabatini, A.Vacca and P.Gans, Talanta, 1974, 21, 53.

36. P.Gans, A.Sabatini and A.Vacca, Inorg.Chim.Acta., 1976, 18, 237.

37. A.Vacca, A.Sabatini and M.Gristina, Coord.Chem.Rev., 1972, 8, 45.

38. E.Teller, " The Persuit of Simplicity ", Pepperdine University Press, 1980.

39. A.M.Corrie, G.K.R.Makar, M.L.D.Touche and D.R.Williams, J.Chem.Soc. Dalton, 1975, 105.

40. G.K.R.Makar, M.L.D.Touche and D.R.Williams, J.Chem.Soc. Dalton, 1976, 1016.

41. D.R.Williams, J.Chem.Soc. Dalton, 1973, 1064.

42. Tutin and Clewer, J.Chem.Soc., 1914, 105, 1846.

43. F.Vorsatz, J.Prak.Chem., 1936, 145, 265.

44. A.Vogel, " Textbook of Quantitative Inorganic Analysis ", 4th Edition, Longmans, New York, 1981.

45. G.Biedermann and J.T.Chow, Acta.Chem.Scand., 1966, 20, 2.

46. G.Schwarzenbach, " Complexiometric Titrations ", 2nd Edition, Methuen, 1967.

47. F.Feigl and V.Anger, " Spot Tests in Inorganic Analysis ", Elsivier, 1972.

48. F.J.C.Rossotti and H.Rossotti, J.Chem.Ed., 1965, 42, 7, 375.

49. R.C.West Ed., "CRC Handbook of Chemistry and Physics ", 63 Edition, CRC PRess, 1982-1983.

50. L.Pauling, " The Nature of the Chemical Bond ", 3rd Edition, Cornell University Press, New York, 1960.

51. D.Dyrssen and I.Hansson, Marine Chem., 1972, 1, 37.

52. C.A.Tyson and A.E.Martell, J.Amer.Chem.Soc., 1968, 90, 3379.

53. R.F.Jameson and W.F.S.Neillie, J.Inorg.Nuclear Chem. 1965, 27, 2623.

54. V.T.Athavale, L.H.Prabhu and D.G.Vartak, J.Inorg.Nucl.Chem., 1966, 28, 1237.

55. R.F.Jameson and W.F.S.Neillie, J.Inorg.Nuclear Chem. 1966, 28, 2667.

56. G.V.Fazakerley, P.W.Linder, R.G.Torrington and M.R.W.Wright, J.Chem.Soc. Dalton, 1979, 1872.

57. J.A.Ibers and W.C.Hamilton Ed., " International tables for X-Ray Crystallography ", Volume 4, Kynoch Press, Birmingham, England, 1974.

58. P.W.Linder, R.G.Torrington and U.A.Seemann, Talanta, 1983, 30, 4, 295.

59. Z.X.Huang, P.M.May, K.M.Quinlan, D.R.Williams and A.M.Creighton, Agents and Actions, In Press.

60. K.Murray, Personal Communications

61. D.K.Nordstrom, A.E.Jenne and J.W.Ball, Chapter 3 in "Chemical Modeling in Aqueous Systems ", A.E.Jenne Ed., ACS Symposium Series, 1978.

62. W.B.White, S.M.Johnson and G.B.Dantzig, J.Chem.Phys., 1958, 28, 5, 751.

63. S.R.Brinkley, J.Chem.Phys., 1947, 15, 2, 107.

64. F.J.Zeleznik and S.Gordon, Industrial and Engineering Chemistry., 1968, 60, 6, 27.

65. L.G.Sillén, Chemistry in Britain, 1967, 291.

66. T.M.Florence, Talanta, 1982, 29, 345.

67. P.M.M.May, P.W.Linder, D.R.Williams, J.Chem.Soc.Dalton, 1977, 588.

68. G.K.R.Makar and D.R.Williams, J.Inorg.Nucl.Chem., 1977, 39, 201.

69. M.C.White, F.D.Baker, R.L.Chaney and A.M.Decker, Plant Physiol., 1981, 67, 301.

70. K.Murray, Ph.D.Thesis, U.C.T., 1982.

71. D.D.Perrin and I.G.Sayce, Talanta, 1967, 14, 833.

72. P.M.M.May, Ph.D. Thesis, UWIST, 1980.

73. J.C.Westall, J.L.Zachary and F.M.M.Morel, Technical note 18, Water Quality Laboratory, M.I.T., 1975.

74. J.C.Little, M.Sc. Thesis, U.C.T., 1984.

75. R.E.McDuff and F.M.M.Morel, Technical Report EQ73-Q2, Keck Laboratory, Caltec, 1973.

76. F.Morel and J.Morgan, Env.Sci.Tech., 1972, 6, 1, 58.

77. D.Dickson, Science, 1984, 227, 34.

78. J.Kielland, Amer.Chem.Soc.J., 1937, 59, 1675.

79. A.H.Truesdell and B.F.Jones, Jour.Research U.S.Geol.Survey, 1974, 2, 2, 233.

80. E.J.Hewitt, " Sand and Water Culture Methods used in the Study of Plant Nutrition ", 2nd Edition, Technical Communication No.22. Commonwealth Agricultural Bureau, Farnham Royal, 1966.

81. E.Epstein, " Mineral Nutrition of Plants: Principle and Perspectives ", Wiley, New York, 1972.

82. L.G.Sillén and A.E.Martell, " Stability Constants of Metal-Ion Complexes ", Spec.Publ.No.17, Chemical Society, London, 1964.

83. W.L.Lindsay, "Chemical Equilibria in Soils", John Wiley and Sons, New York, 1979.

84. L.G.Sillén and A.E.Martell, " Stability Constants of Metal-Ion Complexes ", Spec.Publ.No.25, Chemical Society, London, 1971.

85. E.Högfeldt, " Stability Constants of Metal-Ion Complexes Part A, Inorganic Ligands ", IUPAC, Pergamon Press, 1982.

86. D.G.Perrin, " Stability Constants of Metal-Ion Complexes Part B, Organic Ligands ", IUPAC, Pergamon Press, 1979.

87. A.E.Martell and R.M.Smith, " Critical Stability Constants ", Volume 3, Other Organic Ligands, Plenum Press, New York, 1976.

88. R.M.Smith and A.E.Martell, " Critical Stability Constants ", Volume 4, Inorganic Ligands, Plenum Press, New York, 1976.

89. C.F.Baes Jr. and R.E.Mesmer, " THe Hydolysis of Cations ", John Wiley and Sons, New York, 1976.

90. G.Biedermann and S.Schindler, Acta.Chem.Scand., 1957, 11, 731.

91. J.W.Ball, A.E.Jenne and D.K.Nordstrom, Chapter 25 in " Chemical Modeling in Aqueous Systems ", E.A.Jenne Ed., ACS Symposium Series, 1978.

92. A.P.Schwab and W.L.Lindsay, Soil Sci.Soc.Am.J., 1983,47 201.

93. F.N.Ponamperuma, E.M.Tianco and L.Teresita, Soil Sci., 1976, 103, 374.

94. H.L.E.Madsen, Acta.Chem.Scand., 1970, 24, 1671.

95. D.R.Wier, S.H.Chien and C.A.Black, Soil Sci., 1972, 111(2), 107.

96. R.M.H.Verbeek, H.Staeyer, H.P.Thun and F.Verbeek, J.Chem.Soc. Faraday 1, 1980, 76, 209.

97. J.S.Clark, Cand.J.Chem., 1955, 33, 1696.

98. H.S.Harned and S.R.Scholes Jr., J.Amer.Chem.Soc., 1941, 63, 1706.

99. H.S.Harned and R.Davis Jr., J.Amer.Chem.Soc., 1943, 65, 2030.

100. T.Shedlovsky and D.A.McInnes, J.Amer.Chem.Soc., 1935, 57, 1705.

101. W.Stumm and J.J.Morgan, " Aquatic Chemistry ", John Wiley and Sons, New York, 1981.

102. F.S.Nakayama, Soil Sci., 1972, 113, 6, 456.

103. D.Langmuir, Geochim.Cosmochim.Acta, 1968, 32, 835.

104. R.L.Jacobson and D.Langmuir, Geochim.Cosmochim.Acta, 1974, 38, 301.

105. J.E.Ambler and J.C.Brown, Plant Physiol., 1972, 50, 675.

106. M.C.White, Personal communications.

107. C.M.Johnson, P.R.Stout, T.C.Broyer and A.B.Carlton, Plant Soil, 1957, 8, 337.

108. G.A.Wallace, A.Wallace and M.A.AbouZamZam, J.Pl.Nutr., 1983, 6(6), 545.

109. L.N.Plummer and T.M.L.Wigley, Geochim.Cosmochim.Acta, 1976, 40, 191.

110. L.N.Plummer, T.M.L.Wigley and D.L.Parkhurst, Amer.Jour.Sci., 1978, 278, 179.

111. M.M.Reddy, L.N.Plummer and E.Busenberg, Geochim.Cosmochim. Acta, 1981, 45, 1281.

112. L.N.Plummer, D.L.Parkhurst and T.M.L.Wigley in " Chemical Modeling in Aqueous Systems ", E.A.Jenne Ed., ACS Symposium Series, 1978.

113. P.C.Singer and W.Stumm, Science, 1970, 167, 1121.

114. R.D.Lindberg and D.D.Runnells, Science, 1984, 225, 925.

115. A.Wallace and Collaboraters in " Regulation of the Micronutrient Status of Plants by Chelating Agents and Other Factors ", A.Wallace Ed., Los Angeles, CA, 1971.

116. A.Wallace, R.T.Mueller and G.V.Alexander, Soil Sci., 1978, 126, 6, 336.

117. A.Wallace and G.A.Wallace, J.Pl.Nutr., 1983, 6(6), 541.

118. A.Wallace, J.Pl.Nutr., 1980, 2(1-2), 163.

119. A.Wallace, R.T.Mueller and E.M.Romney, Commun.Soil Sci. Plant Anal., 1976, 7, 93.

120. A.Wallace, G.A.Wallace and G.V.Alexander, J.Pl.Nutr., 1983, 6(6), 507.

121. A.D.Halvorson and W.L.Lindsay, Soil Sci.Soc.Amer.J., 1977, 41, 531.

122. A.P.Schwab and W.L.Lindsay, Soil Sci.Soc.Amer.J., 1983, 47, 217.

123. J.C.Brown, L.O.Tiffin and R.S.Holmes, Plant Physiol., 1960, 35, 878.

124. D.J.Linehan and H.Shepherd, Plant and Soil, 1979, 52, 281.

125. D.G.Hill-Cottingham and C.P.Lloyd-Jones, J.Exp.Bot., 1965, 16, 47, 233.

126. R.A.Jeffreys and A.Wallace, Agro.J., !968, 60, 613.

127. A.Jugsujinda and W.H.Patrick Jr., Agro.J., 1977, 69, 705.

128. K.Venkat Raju and H.Marschner, Z.Pflansenernähr.Bodenkd., 1981, 144, 339.

129. V.Römheld, H.Marschner and D.Kramer, J.Pl.Nutr., 1982, 5(4-7), 489.

130. W.L.Lindsay and W.A.Norvell, Soil Sci.Soc.Amer.J., 1969, 33, 62.

131. F.A.Cotton and G.Wilkinson, " Advanced Inorganic Chemistry", 4th Edition, John Wiley and Sons, New York, 1980.

132. G.E.Jackson, P.M.May and R.R.Williams, J.Inorg.Nucl.Chem., 1978, 40, 1227.

133. G.E.Jackson, P.M.May and D.R.Williams, J.Inorg.Nucl.Chem., 1981, 43, 825.

134. T.M.Florence and G.E.Batley, CRC Crit.Rev.Anal.Chem., August 1980, 219.

135. A.Wallace, J.Pl.Nutr., 1982, 5(4-7), 277.

136. J.Benton Jones Jr., J.Pl.Nutr., 1982, 5(8), 1003.

137. J.C.Brown, Chapter 5 in " Bioinorganic Chemistry II ", K.N.Raymond Ed., A.C.S., Washington, D.C., 1976.

138. G.W.Bates, J.Pl.Nutr., 1982, 5(4-7), 269.

139. S.D.Nelson et al, Eds., J.Pl.Nutr., 1982, Iron Symposium issue, 5(1-6).

140. P.M.May, M.Sc. Thesis, University of Cape Town, 1975.

141. S.M.Elgawhary, W.L.Lindsay and W.D.Kemper, Soil Sci.Soc. Amer.Proc., 1970, 34, 66.

143. M.C.White, A.M.Decker and R.L.Chaney, Plant Physiol., 1981, 67, 292.